

Supplementary Materials

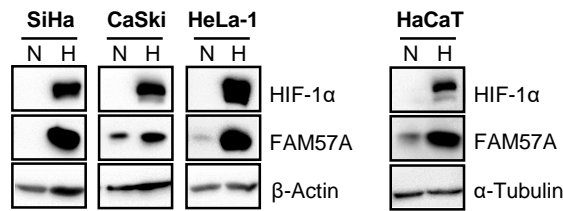
FAM57A (Family with sequence similarity 57 member A) is a cell density-regulated protein and promotes the proliferation and migration of cervical cancer cells

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Supplementary Figures S1-S6

Related to Figure 1a:



Original blots and quantification of blots corresponding to Figure 1a:

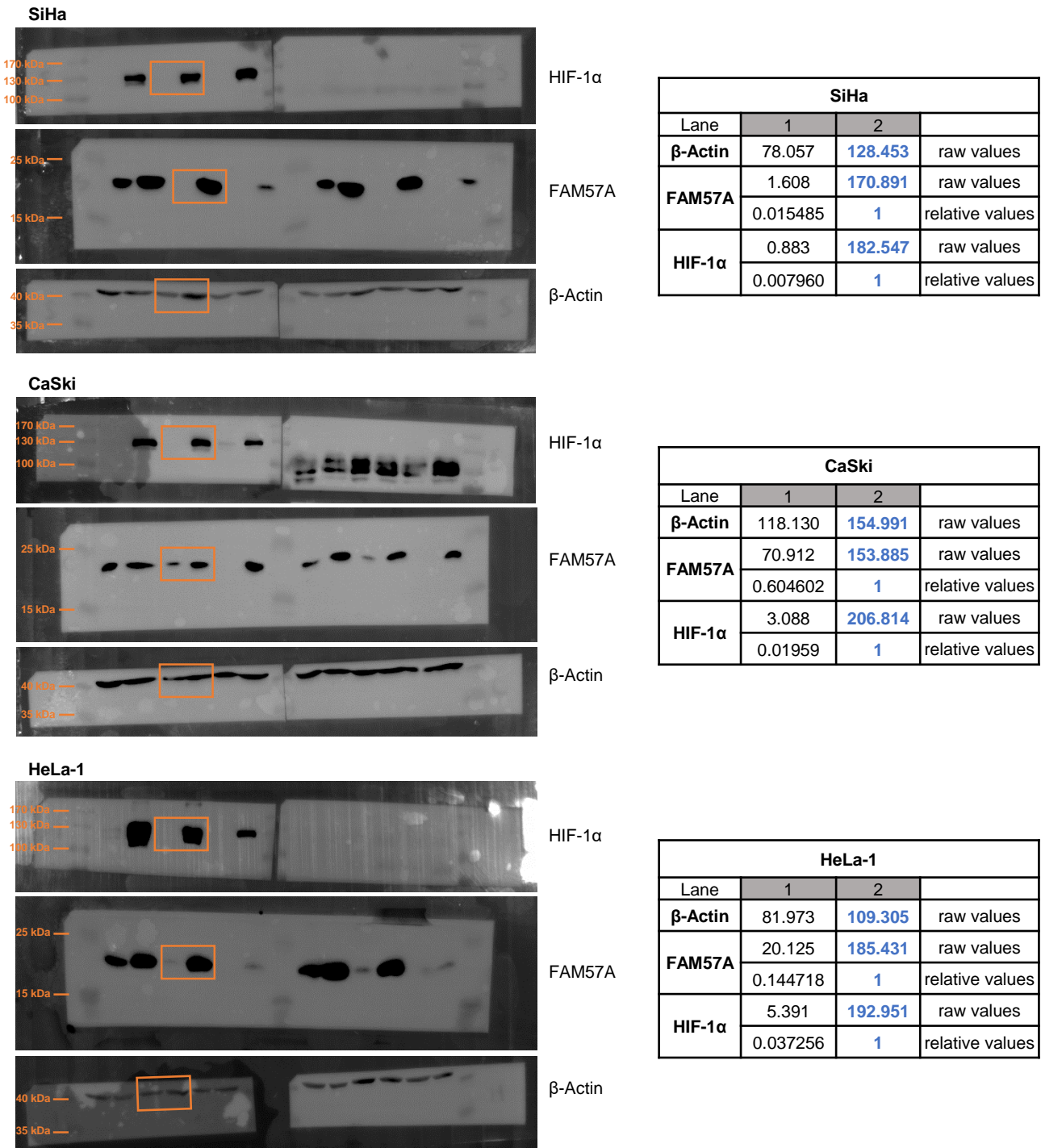
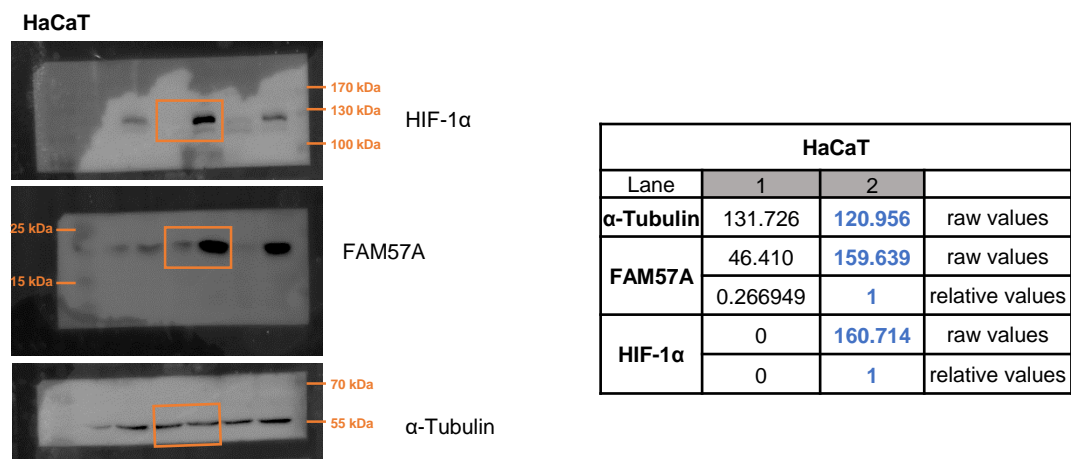
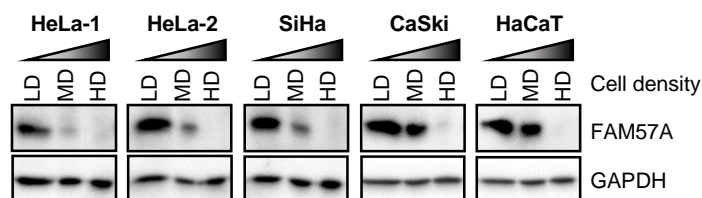


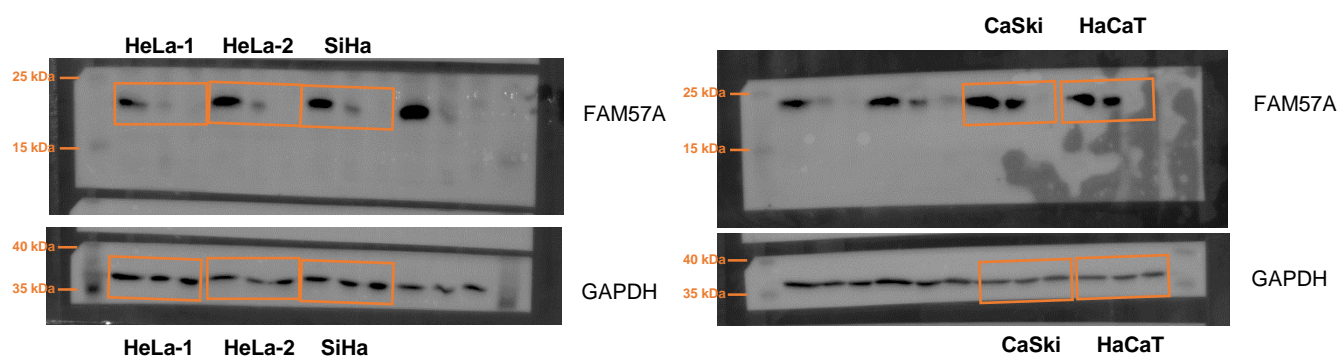
Figure S1. Original blots and quantification of immunoblots. Uncropped original immunoblots including molecular weight markers are depicted. Immunoblots were quantified densitometrically with background subtraction and inversion (raw values), and were normalized to loading control and subsequently to the value in blue (relative values). Continued on next pages.



Related to Figure 2a:



Original blots and quantification of blots corresponding to Figure 2a:

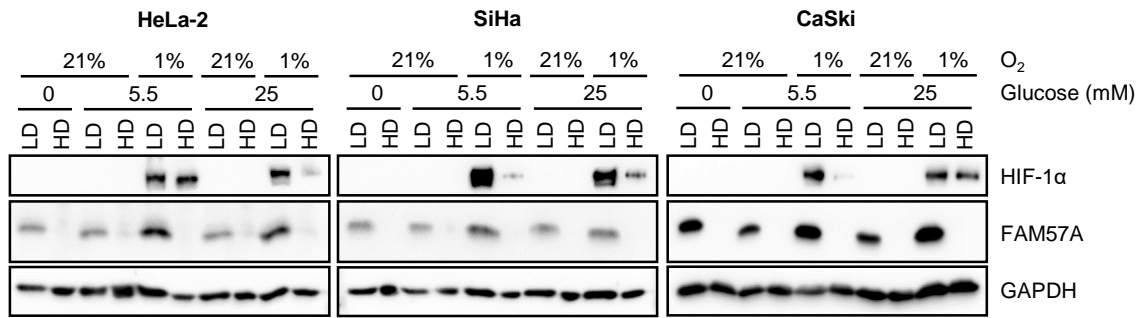


	HeLa-1			HeLa-2			SiHa			
Lane	1	2	3	1	2	3	1	2	3	
GAPDH	169.365	127.143	138.009	129.886	87.130	112.965	162.34	148.830	173.932	raw values
FAM57A	131.580	27.730	9.924	166.187	49.407	1.444	157.294	53.556	2.986	raw values
	1	0.280732	0.092558	1	0.443186	0.009991	1	0.371391	0.017718	relative values

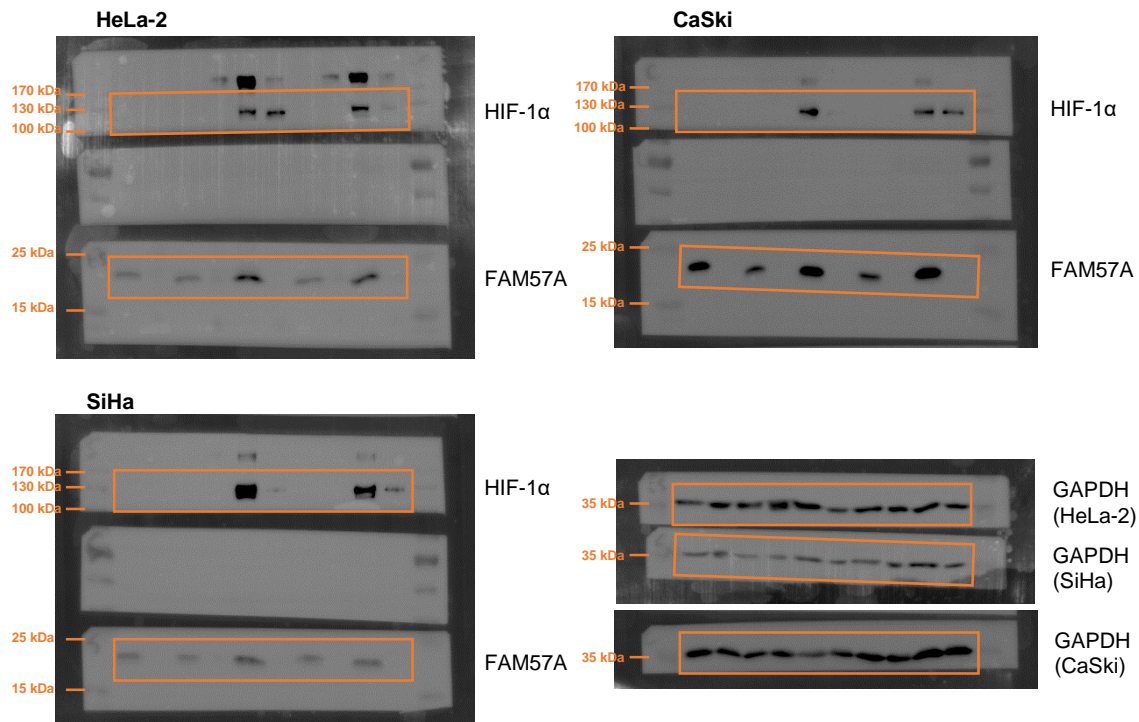
	CaSki			HaCaT			
Lane	1	2	3	1	2	3	
GAPDH	133.659	128.594	141.471	124.573	117.447	118.540	raw values
FAM57A	191.790	132.016	12.749	147.683	101.418	2.158	raw values
	1	0.715448	0.062803	1	0.728394	0.01536	relative values

Figure S1 continued.

Related to Figure 3a:



Original blots and quantification of blots corresponding to Figure 3a:



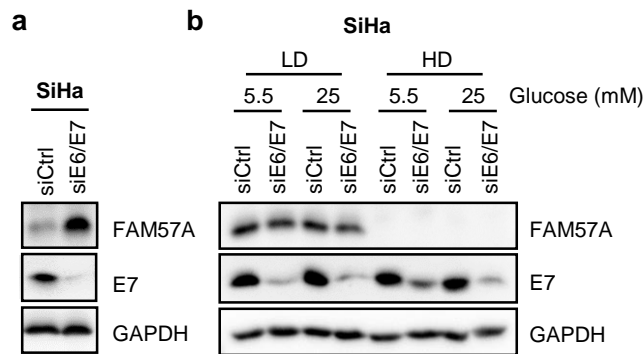
HeLa-2											
Lane	1	2	3	4	5	6	7	8	9	10	
GAPDH	90.048	112.171	117.124	147.887	145.331	69.567	110.596	101.320	115.258	96.967	raw values
FAM57A	40.187	7.572	42.490	15.902	101.418	9.179	40.126	18.048	84.910	7.424	raw values
	1	0.151258	0.812885	0.240941	1.563671	0.295652	0.812971	0.399137	1.650731	0.171555	relative values
HIF-1α	0	0	0	0.106	146.507	146.956	0.293	0	145.272	27.781	raw values
	0	0	0	0.000711	1	2.095482	0.002628	0	1.25029	0.2842	relative values

SiHa											
Lane	1	2	3	4	5	6	7	8	9	10	
GAPDH	114.796	130.396	80.879	83.362	147.175	91.941	138.362	120.125	169.358	110.679	raw values
FAM57A	42.502	3.864	30.499	7.544	74.465	8.348	41.439	0.726	57.117	1.077	raw values
	1	0.080037	1.018514	0.244428	1.366582	0.245240	0.808928	0.016324	0.910913	0.026283	relative values
HIF-1α	0.340	0	0	0.560	195.961	12.582	0.510	0	151.628	39.829	raw values
	0.002224	0	0	0.005045	1	0.102779	0.002768	0	0.672416	0.270270	relative values

CaSki											
Lane	1	2	3	4	5	6	7	8	9	10	
GAPDH	149.955	133.417	121.25	127.968	100.747	126.298	154.201	144.259	182.193	177.150	raw values
FAM57A	136.289	2.136	82.829	2.733	165.96	1.497	102.789	2.587	189.389	1.143	raw values
	1	0.017615	0.751624	0.023498	1.812473	0.013041	0.733432	0.019731	1.143729	0.007099	relative values
HIF-1α	0	0	0	0.490	146.930	8.068	0	0	125.582	99.955	raw values
	0	0	0	0.002626	1	0.043802	0	0	0.472626	0.386888	relative values

Figure S1 continued.

Related to Figure 4:



Original blots and quantification of blots corresponding to Figure 4:

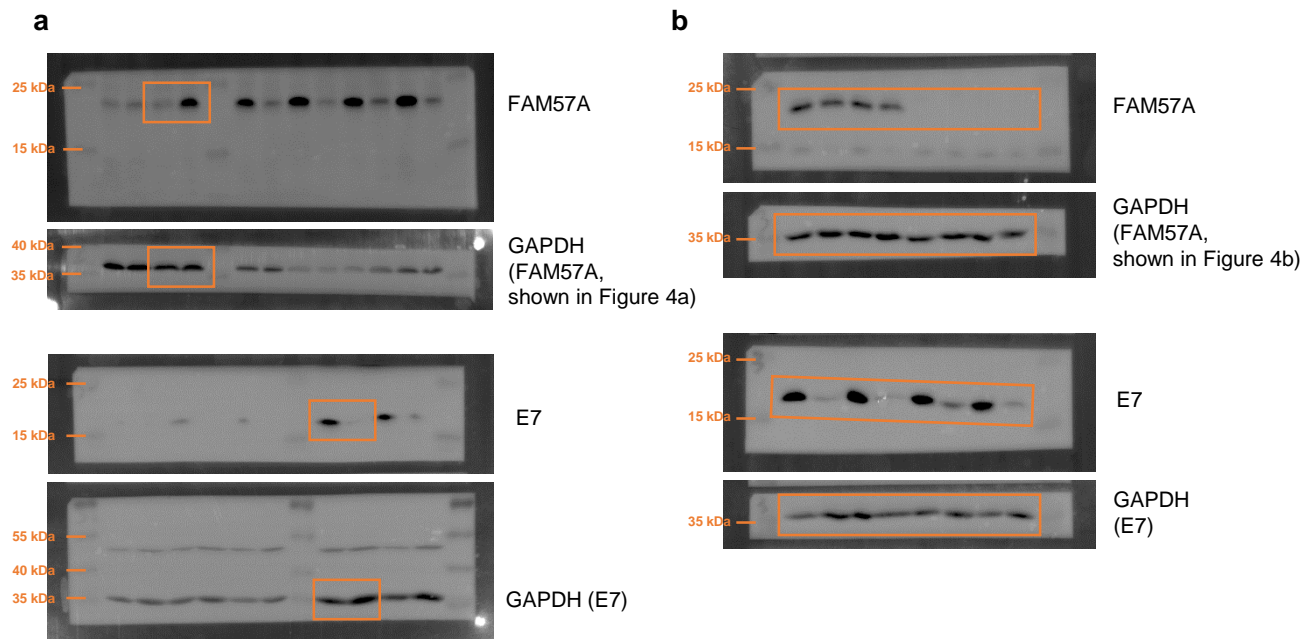
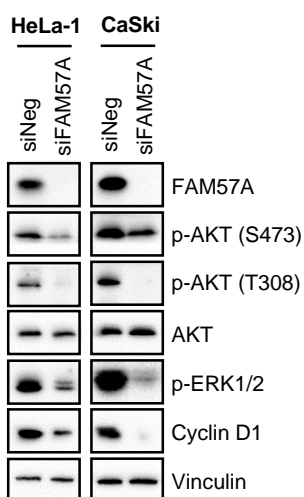


Figure 4a			
Lane	1	2	
GAPDH (FAM57A)	153.212	144.353	raw values
FAM57A	34.507	154.961	raw values
	1	4.766309	relative values
GAPDH (E7)	148.077	182.875	raw values
E7	151.073	16.294	raw values
	1	0.087332	relative values

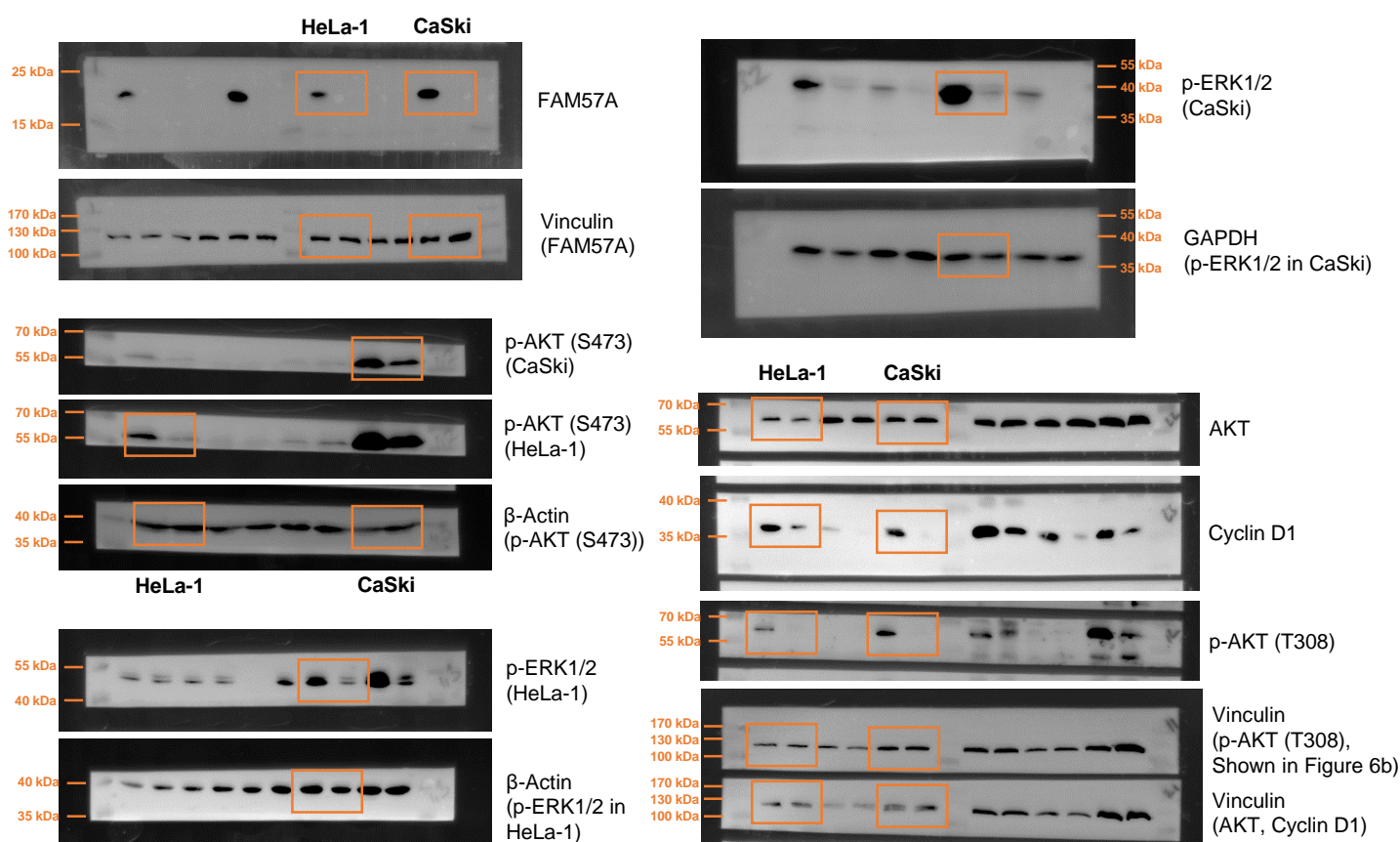
Figure 4b									
Lane	1	2	3	4	5	6	7	8	
GAPDH (FAM57A)	118.018	139.078	134.668	145.474	117.548	131.395	122.323	114.833	raw values
FAM57A	121.186	98.321	104.624	87.589	0	0	0.030	0	raw values
	1	0.688468	0.756594	0.586354	0	0	0.000239	0	relative values
GAPDH (E7)	127.043	176.172	179.62	156.905	153.151	166.974	131.019	156.848	raw values
E7	144.869	28.366	146.299	29.385	140.862	68.000	137.888	32.128	raw values
	1	0.141201	0.714269	0.164234	0.806583	0.357137	0.922927	0.179630	relative values

Figure S1 continued.

Related to Figure 6b:



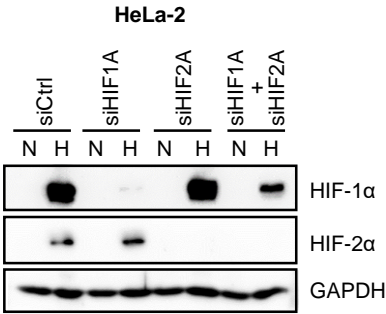
Original blots and quantification of blots corresponding to Figure 6b:



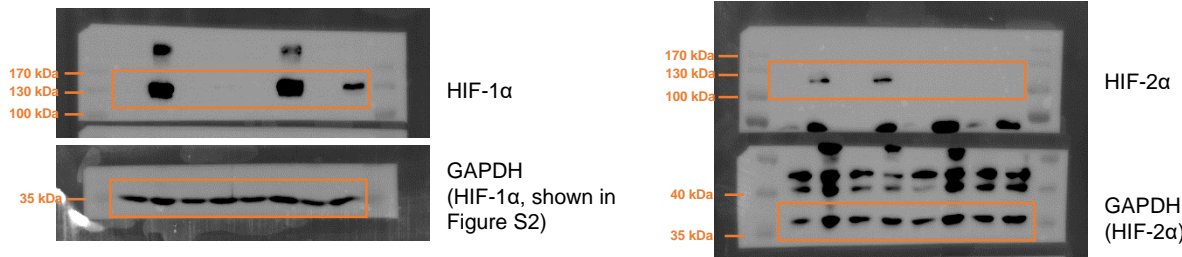
	HeLa-1		CaSki				HeLa-1		CaSki		
Lane	1	2	1	2		Lane	1	2	1	2	
Vinculin (FAM57A)	110.536	120.756	115.962	158.590	raw values	Vinculin (AKT + Cyclin D1)	102.000	115.682	120.338	132.588	raw values
FAM57A	152.229	3.286	179.214	1.758	raw values	AKT	159.095	138.642	140.308	163.010	raw values
	1	0.019759	1	0.007173	relative values		1	0.768374	1	1.054461	relative values
β-Actin (p-AKT (S473))	124.452	136.512	99.423	119.546	raw values	Cyclin D1	170.545	71.645	171.416	11.416	raw values
	1	0.374553	1	0.612687	relative values		1	0.370409	1	0.060445	relative values
p-AKT (S473)	179.650	73.809	193.721	142.713	raw values	β-Actin/GAPDH (p-ERK1/2)	193.431	156.523	145.205	115.452	raw values
	1	0.374553	1	0.612687	relative values		1	0.264756	1	0.092055	relative values
Vinculin (p-AKT (T308))	131.134	132.705	164.795	150.136	raw values	p-ERK1/2	119.040	25.503	172.531	12.628	raw values
p-AKT (T308)	1	0.087129	1	0.022291	relative values						

Figure S1 continued.

Related to Figure S2:



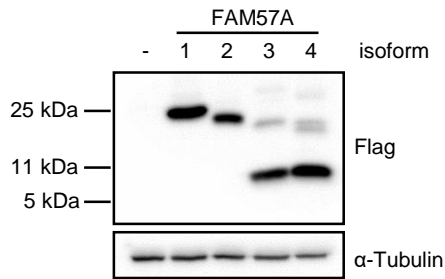
Original blots and quantification of blots corresponding to Figure S2:



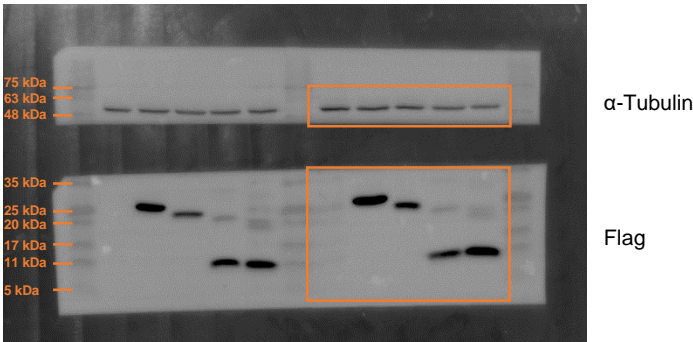
	HeLa-2								
Lane	1	2	3	4	5	6	7	8	
GAPDH (HIF-1α)	148.679	176.386	143.668	168.793	152.928	184.325	147.015	159.760	raw values
HIF-1α	2.147	196.033	1.473	5.310	0.382	207.190	1.944	92.412	raw values
	0.012993	1	0.009225	0.028306	0.002248	1.011392	0.011898	0.520469	relative values
GAPDH (HIF-2α)	93.614	157.210	125.762	128.547	106.816	174.701	129.632	156.884	raw values
HIF-2α	0.842	121.187	0.871	151.770	1.549	0.641	1.057	0	raw values
	0.011668	1	0.008984	1.53161	0.018812	0.00476	0.010578	0	relative values

Figure S1 continued.

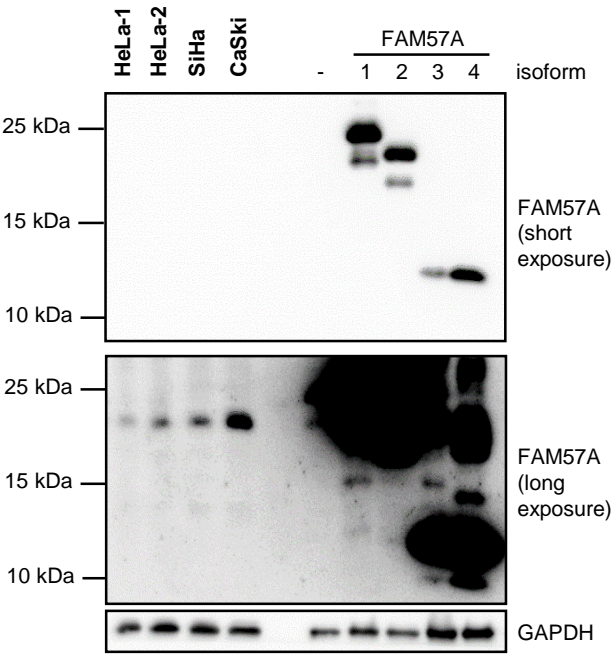
Related to Figure S3a:



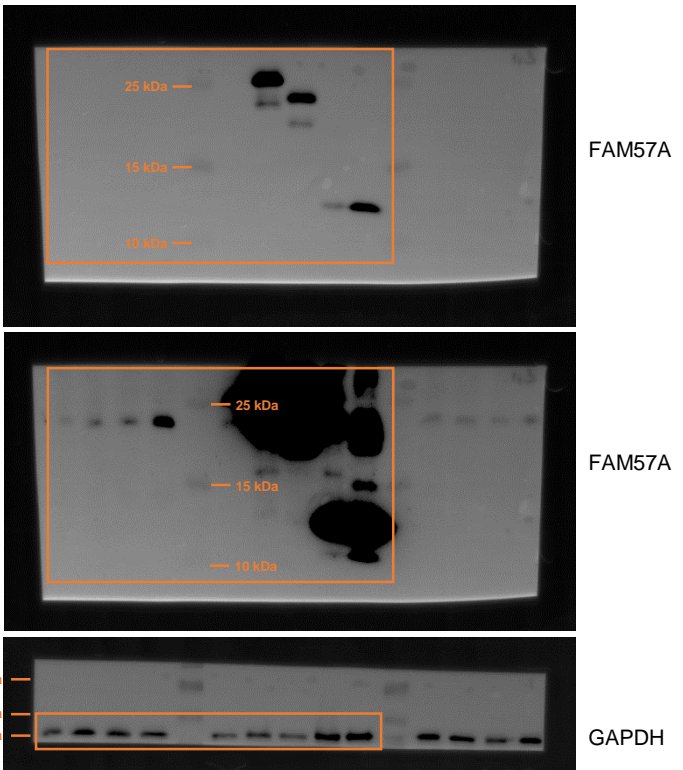
Original blots corresponding to Figure S3a (right panel):



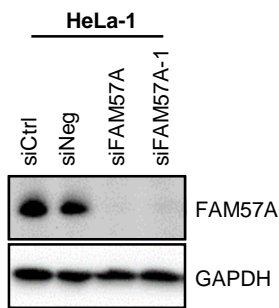
Related to Figure S3b:



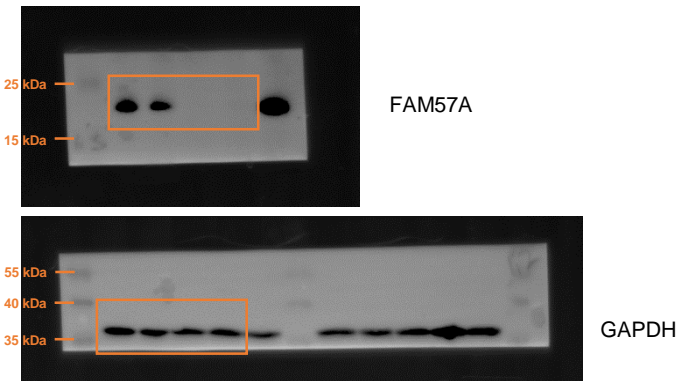
Original blots corresponding to Figure S3b (right panel):



Related to Figure S3d:



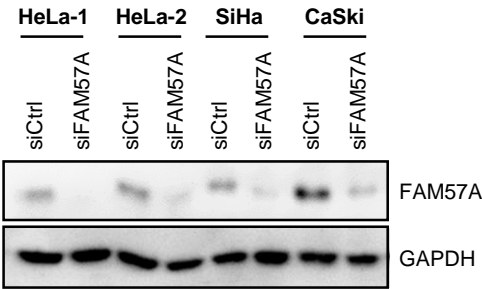
Original blots and quantification of blots corresponding to Figure S3d (right panel):



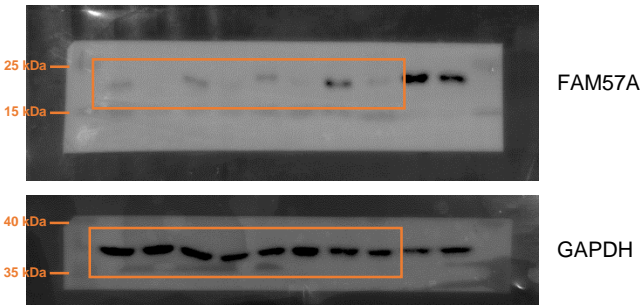
HeLa-1					
Lane	1	2	3	4	
GAPDH	198.047	178.569	173.828	181.55	raw values
FAM57A	154.324	128.365	2.945	4.769	raw values
	1	0.922519	0.021742	0.033711	relative values

Figure S1 continued.

Related to Figure S6c:



Original blots and quantification of blots corresponding to Figure S6c:



	HeLa-1		HeLa-2		SiHa		CaSki		
Lane	1	2	3	4	5	6	7	8	
GAPDH	99.200	129.217	113.500	92.683	139.182	132.067	128.550	98.267	raw values
FAM57A	30.874	10.081	83.852	14.437	38.443	8.261	43.594	14.183	raw values
	1	0.250670	1	0.210843	1	0.226467	1	0.425604	relative values

Figure S1 continued.

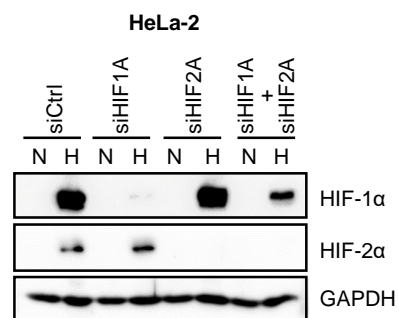


Figure S2. Immunoblot analyses measuring HIF-1α and HIF-2α protein levels following silencing *HIF1A* (siHIF1A) or *HIF2A* (siHIF2A) expression, either alone or in combination. siCtrl, control siRNA; GAPDH, loading control.

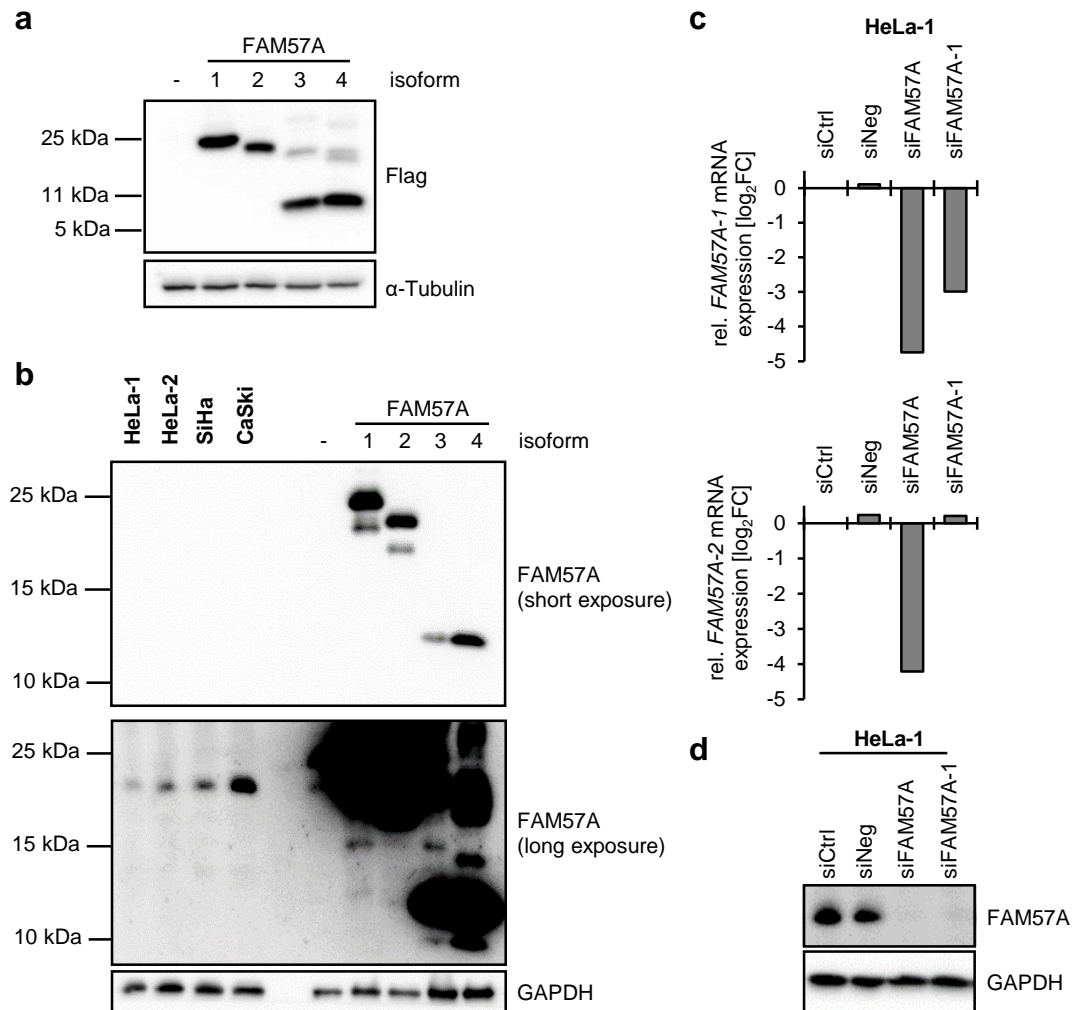


Figure S3. FAM57A antibody validation and detection of endogenous FAM57A-1 expression in cervical cancer cells. **(a)** The four predicted FAM57A isoforms (FAM57A-1, FAM57A-2, FAM57A-3, FAM57A-4) were ectopically overexpressed as Flag-tagged proteins in HeLa-1 cells and analyzed by immunoblot using an anti-Flag antibody. Size markers are indicated. α -Tubulin, loading control. **(b)** Endogenous FAM57A protein expression in HeLa-1, HeLa-2, SiHa and CaSki cells (left part of the figure) as well as ectopically overexpressed FAM57A in HeLa-1 cells (right part of the figure) were analyzed by immunoblot, employing the anti-FAM57A antibody generated in this work. Upper panel: short exposure, lower panel: long exposure of the same blot. Size markers are indicated. GAPDH, loading control. **(c, d)** In order to determine whether the endogenous FAM57A signal in HPV-positive cancer cells relates to the 257 amino acid FAM57A-1 (CT120A) or to the only 32 amino acid shorter FAM57A-2 (CT120B) isoform, we utilized an isoform-specific siRNA (siFAM57A-1). It efficiently downregulates the transcript coding for the FAM57A-1 isoform (upper panel), but does not affect the levels of the FAM57A-2-encoding transcript (lower panel) **(c)**. Immunoblot analyses show that the endogenous FAM57A signal in HeLa-1 cells is efficiently downregulated by siFAM57A-1 **(d)**, indicating that it is derived from the FAM57A-1 isoform. siCtrl and siNeg, control siRNAs; GAPDH, loading control.

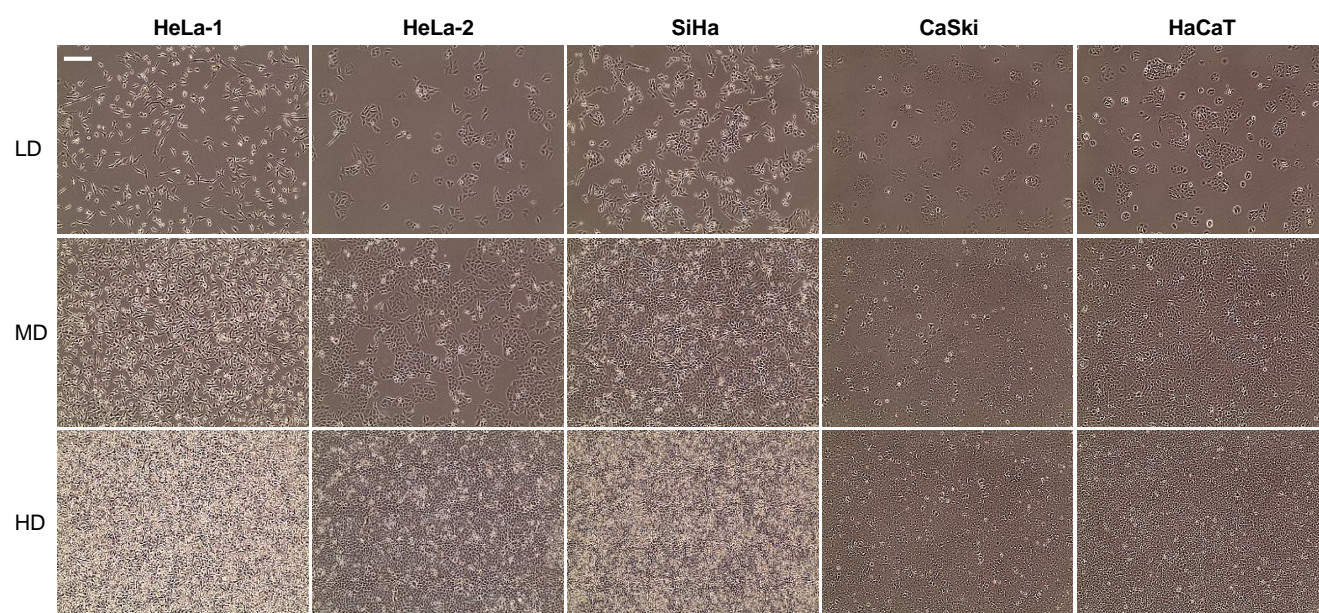


Figure S4. Microscopic visualization of different cell densities before harvesting the cells for measuring FAM57A expression levels by immunoblot analyses (relates to Figure 2). LD: low density; MD: medium density; HD: high density. A size marker is indicated in the upper left panel (scale bar: 200 μ m).

5'...CAGAGTCCTGCTCTGTGGCCCAGGCTGGAGTGCAGTGG**CGTG**ATCTCGGCTCACTGCAACCTCT
 GCCTCCCAGGTTCAAGCGATTCTCCTGCCTCAGCCTCCAGAGTAGCTGGGACTACAGACAGGCACCAC
 CACACCTGGCTAATTTTGTATTTTGTAGTAGAGACGGGGTTTACCCTGGTGGTGCAGGCTGGTCTCGAAC
 TCCTGACGTCAAGTGATCTGCCCCGCTCGGCCTCCCCAAGTGCTGGGGTTACAGGCGCGCCCGACCC
 CGATACTTTTAAATAACGA**CACGT**TGTGAACAACACCACAGTCCCTACGCCTCTGGATGGTGTCTCCGG
 ATAGATCCAAAGCCGAGAGGCAGTAAGAGCCTGCAGCCTCCACAGTCCGCCTCCTGGTGCAGGTGGA
 GAGGGGCTGCTGGAGGCCCGGACCT**CACGC**TTCTGAGGGCCACCCCGAGCGGCTGCAGCCGC
 TGGCGGCACCGGGAAAAGCATCTCGGCCGTGCGGGGAGAGGCCACATCCTGAGGCCTCCACCGAGA
 AGCACCAGGGCTTGGTGGACGGGGCCCGCAACCCAGGGCCA**CACGC**CGCCTCCCTCCTTCCTTCCC
 CGCGGGCCTCGCCCCACCCCGAGGGGGTTCTACCTGGCTTCTCGGCCCGCGCCACCCCGCC
 CCTTCGCCCCGGGGCCGGCGAGAAGAGCAGGTGCGGGGAGGGGCGCGTCCGCTGACGGAACAGAAGCC
 GGGAGCCGCGGCCCTTCCCGCCTCCGCCTGGGCCCGCGGCCGCTCGGGAGGGAGCGGCGGACTC
 GGAACGCGCGCGCGCCGCGGCCCGGCCACAGCCGCCGCTCGGTCCCCGCCTCCGCCCCCGCCCA
 CCCGCCCGCCGGGGACGCCGGGGAACCTCCGGGCCAGGGACCGCGAGAGACCCCGCCCCGCC
 TATCGCAGCCGCCGGGCCTGGT**CACGC**GCAGAGCCGCGCCGCGGGATCGGGGCCAGCGCCGGCG
 GAGGCGGGACGGGGACGGGGCGGAGGGTTGAAATCGCGCGGCCGGGCCGGGGCGCGCC**GAGCC**
 GAACCCAGC**CACGC**GGCGCCAGCGAGGCGGCCGGACCCGCAGCCCCGATGCTGCTGACG...-3'

Figure S5. Potential HIF-1 binding sites in the promoter region (nucleotides -1056 to +64) of the *FAM57A* gene. Core consensus binding sequences for HIF-1 (5'- A/G-C-G-T-G -3' or its complementary sequence 5'- C-A-C-G-T/C -3') are highlighted in bold. The predicted transcriptional start site is marked in bold and italicized, the underlined sequence corresponds to the start of the coding sequence [9].

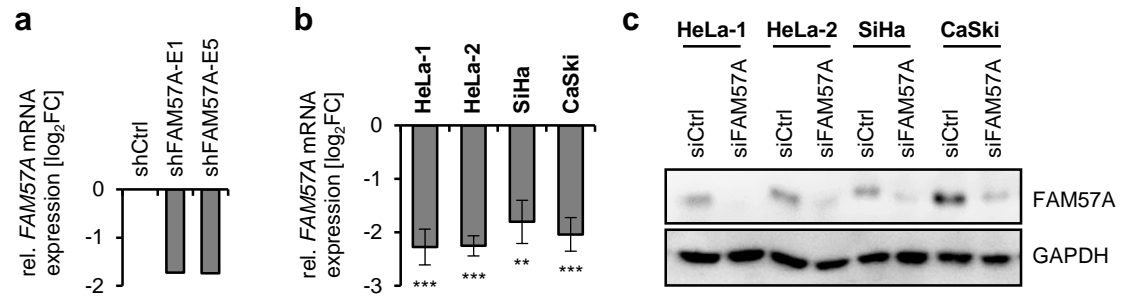


Figure S6. Validation of shRNAs and siRNAs used for *FAM57A* silencing. **(a)** qRT-PCR analyses of *FAM57A* transcript levels upon transfection of HeLa-1 cells with pSUPER plasmids expressing two different *FAM57A*-targeting shRNAs (shFAM57A-E1 or shFAM57A-E5). shCtrl: control shRNA. Shown are the \log_2FC of the *FAM57A* levels following *FAM57A* silencing relative to shCtrl-expressing cells ($\log_2FC=0$). **(b)** Validation of siFAM57A (equimolar pool of siFAM57A-E1 and siFAM57A-E5) in HPV-positive cancer cell lines at the transcript level by qRT-PCR. Shown are the \log_2FC values of mean *FAM57A* transcript levels of siFAM57A-transfected cells relative to control siRNA (siCtrl)-transfected cells ($\log_2FC=0$) with standard deviations from at least 3 independent experiments for each cell line. Statistically significant differences to the respective siCtrl-transfected cells are determined by one-way ANOVA, **: $P < 0.01$, ***: $P < 0.001$. **(c)** Corresponding analyses of FAM57A protein levels by immunoblot analysis under the treatment conditions outlined in subfigure (b). siCtrl, control siRNA; GAPDH, loading control.