Supplement: **Differential Gene Expression Induced By Different TLR Agonists In A549 Lung Epithelial Cells Is Modulated By CRISPR Activation Of TLR10**

1. sgRNA sequences and primers for amplification of the target region

1.1 Spacer sequences

Table S1. Spacer sequences

sgRNA1: caguagcaugcagaagaggg
sgRNA2: gcuaaagcccgcaagaggcu
sgRNA3: gcguggaugauucguugaca
sgRNA4: uccucagaucaauagcccca

1.2 Target region specific primers

Table S2. Target region specific primers

	Forward primer sequence	**Reverse primer sequence**
ACT1-vitro	gccagttggtgagcgcctgtag	ccacggcttgcactctctcagc
ACT2-vitro	agcactttgggaggccgagaca	ccatccgccctcttctgcatgc
ACT3-vitro	catgcagaagagggcggatggg	acccacgctctcaagcatctgc
ACT4-vitro	gctgagagagtgcaagccgtgg	gcagaggttaacatacgctgtgggg

2. Validation of the sgRNAs

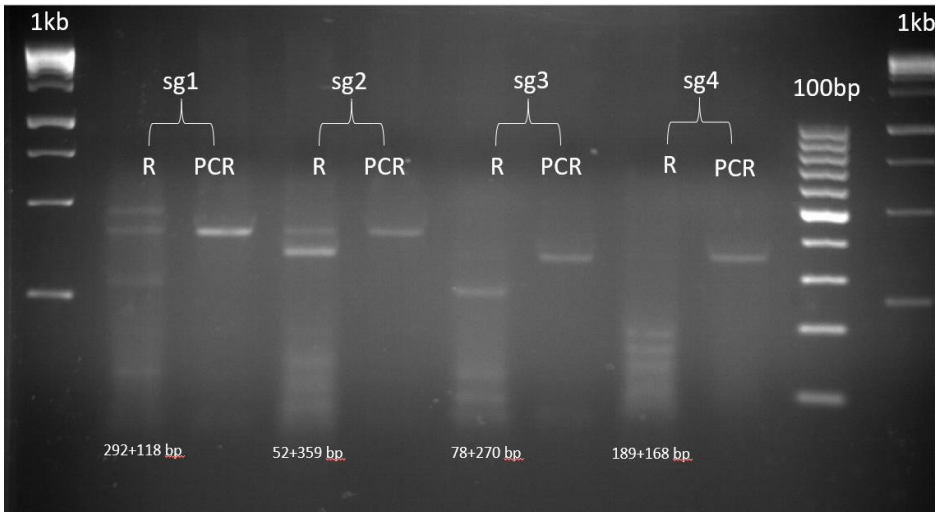

Figure S1. sgRNA validation in vitro with RNP complex. Successful binding of sgRNA and cutting of the double strand with Cas9 can be seen as digested PCR fragments.

3. Affect of overexpressed TLR10 on other membrane or cytoplasmic receptors

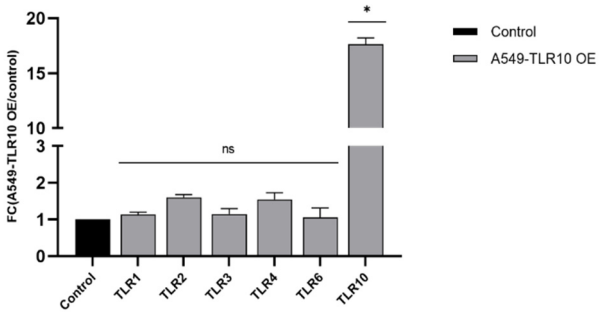

Figure S2. Effect of overexpressed TLR10 on expression of TLR2 subfamily, TLR3 and TLR4. Differential gene expression of the TLRs in A549 cells after overexpression of TLR10. Control samples were co-transfected with empty pGGa-select vector and dCas9-VPR. Data are means ± SD of three different experiments completed in triplicates, *$p < 0.05$, ns= not significant.

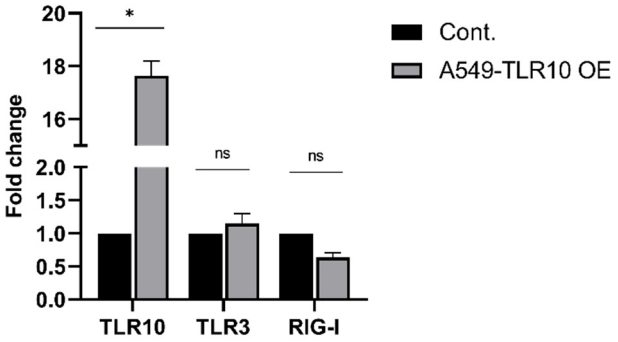

Figure S3. Basal expression of TLR3 and RIG-I. Differential gene expression of the TLR10, TLR3 and RIG-I in A549 cells after overexpression of TLR10. Control samples were co-transfected with empty pGGa-select vector and dCas9-VPR. Data are means ± SD of three different experiments completed in triplicates, *$p < 0.05$.

4. Differential expression of immune associated genes in the immunostimulated A549 cells

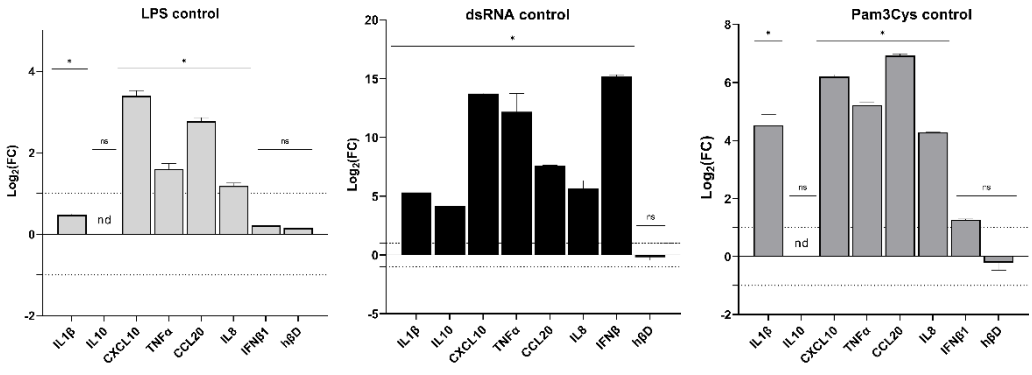

Figure S4. Differential expression of immune associated genes in the immunostimulated A549 cells. Differential gene expression (log2(FC)) of the TLRs and immune mediators in A549 cells after challenge with the different ligands, normalized to unchallenged A549 cells. Cells were stimulated with dsRNA (10 μg/ml), LPS (50 ng/ml) or Pam3Cys (50 ng/ml) for 4 hr. Data are means ± SD of three different experiments completed in triplicates, *$p < 0.05$; ns= not significant, nd= not detected.

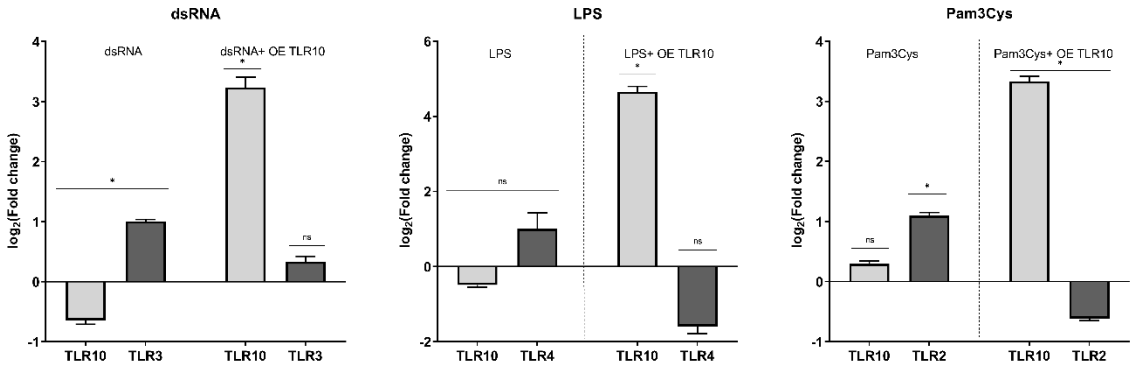

Figure S5. Differential expression of TLR10 and TLR specific for each ligand. The panel shows differential expression (log2(FC)) of TLR10 and TLR specific with each ligand, in the stimulated A549 cells and A549-TLR10 OE. Cells were stimulated with dsRNA (10 μg/ml), LPS (50 ng/ml) or Pam3Cys (50 ng/ml) for 4 hr. Data are means ± SD of three different experiments completed in triplicates, *$p < 0.05$, ns= not significant.

5. Results from pre-designed qPCR profiling arrays

Table S3. Expression analysis of genes related to the TLR biological pathway

UniGene	GenBank	Symbol	log(A549-OE TLR10 + dsRNA)	log(A549-OE TLR10 + LPS)	log(A549-OE TLR10 + Pam3Cys)
Hs.159494	NM_000061	BTK	0,011	0,145	0,520
Hs.599762	NM_001228	CASP8	0,342	-1,065	0,230
Hs.303649	NM_002982	CCL2	0,612	-1,815	-0,540
Hs.163867	NM_000591	CD14	3,827	1,345	1,100
Hs.87205	NM_005582	CD180	NA	-1,435	NA
Hs.838	NM_005191	CD80	-0,698	1,255	-0,140
Hs.171182	NM_006889	CD86	NA	-0,565	-0,180
Hs.198998	NM_001278	CHUK	-1,580	-0,165	-0,180
Hs.236516	NM_014358	CLEC4E	1,792	-0,615	0,730
Hs.1349	NM_000758	CSF2	-6,424	0,015	-3,190
Hs.2233	NM_000759	CSF3	-0,657	0,725	-1,660
Hs.632586	NM_001565	CXCL10	3,601	-0,695	3,410
Hs.515146	NM_016581	ECSIT	-1,230	0,925	0,410
Hs.131431	NM_002759	EIF2AK2	-0,360	-1,485	-0,230
Hs.181128	NM_005229	ELK1	1,564	3,265	-1,050
Hs.86131	NM_003824	FADD	2,849	0,165	0,300
Hs.728789	NM_005252	FOS	0,816	-0,115	0,520
Hs.593339	NM_002128	HMGB1	0,345	0,755	1,000
Hs.37003	NM_005343	HRAS	-2,142	-0,295	0,780
Hs.728810	NM_005345	HSPA1A	2,299	1,765	0,000
Hs.595053	NM_002156	HSPD1	3,137	0,005	1,140
Hs.37026	NM_024013	IFNA1	-3,028	0,825	-1,820
Hs.93177	NM_002176	IFNB1	2,170	0,405	1,730
Hs.856	NM_000619	IFNG	-6,354	NA	NA

Hs.597664	NM_001556	IKBKB	-1,266	-0,255	-0,480
Hs.193717	NM_000572	IL10	-3,923	NA	1,420
Hs.673	NM_000882	IL12A	-1,559	-0,235	0,800
Hs.1722	NM_000575	IL1A	1,169	-0,655	-1,770
Hs.126256	NM_000576	IL1B	1,425	-1,255	-1,360
Hs.89679	NM_000586	IL2	NA	NA	NA
Hs.654458	NM_000600	IL6	-1,618	0,255	-2,940
Hs.624	NM_000584	IL8	1,895	-1,155	-0,280
Hs.522819	NM_001569	IRAK1	NA	2,595	2,810
Hs.449207	NM_001570	IRAK2	-0,728	0,705	-0,960
Hs.138499	NM_016123	IRAK4	0,007	-0,675	-0,890
Hs.436061	NM_002198	IRF1	-1,636	0,325	-0,310
Hs.75254	NM_001571	IRF3	-3,622	-0,635	0,660
Hs.714791	NM_002228	JUN	1,669	-0,565	-1,000
Hs.36	NM_000595	LTA	-0,901	0,515	-1,980
Hs.653138	NM_004271	LY86	-1,499	-1,255	NA
Hs.660766	NM_015364	LY96	-2,998	-0,975	0,740
Hs.514012	NM_002756	MAP2K3	-6,139	0,555	1,950
Hs.514681	NM_003010	MAP2K4	-1,572	-0,695	-0,270
Hs.657756	NM_005921	MAP3K1	-1,038	-0,985	-0,690
Hs.644143	NM_003188	MAP3K7	1,943	-0,525	-0,580
Hs.431550	NM_004834	MAP4K4	-0,656	-0,915	0,200
Hs.138211	NM_002750	MAPK8	-1,179	-1,475	-0,760
Hs.207763	NM_015133	MAPK8IP3	-0,478	0,265	-0,480
Hs.82116	NM_002468	MYD88	-1,199	0,295	-2,500
Hs.654408	NM_003998	NFKB1	-1,643	-0,465	-0,730
Hs.73090	NM_002502	NFKB2	-2,066	-0,195	-1,150
Hs.81328	NM_020529	NFKBIA	0,667	-0,635	-0,910
Hs.2764	NM_005007	NFKBIL1	1,232	1,505	1,170
Hs.530539	NM_006165	NFRKB	-0,007	0,515	-0,130
Hs.591667	NM_003298	NR2C2	0,287	-0,955	-0,910
Hs.7886	NM_020651	PELI1	2,026	-0,915	-0,190
Hs.103110	NM_005036	PPARA	3,199	-0,635	-0,290
Hs.570274	NM_003690	PRKRA	-1,827	-1,225	-0,190
Hs.196384	NM_000963	PTGS2	-2,485	-0,445	0,470
Hs.631886	NM_002908	REL	-0,684	-0,505	-0,320
Hs.502875	NM_021975	RELA	-1,670	-1,295	-0,970
Hs.103755	NM_003821	RIPK2	-0,839	-0,935	0,240
Hs.532781	NM_015077	SARM1	0,530	-1,285	-1,920
Hs.501624	NM_021805	SIGIRR	-0,426	0,435	-0,270
Hs.507681	NM_006116	TAB1	-0,222	-0,225	-0,770
Hs.505874	NM_013254	TBK1	1,128	1,545	1,740
Hs.29344	NM_182919	TICAM1	2,534	-2,105	-0,370
Hs.710895	NM_021649	TICAM2	2,191	-1,005	-0,820

Hs.537126	NM_001039661	TIRAP	0,767	1,015	-0,240
Hs.654532	NM_003263	TLR1	1,179	-0,945	-0,260
Hs.519033	NM_003264	TLR2	NA	-0,255	-0,140
Hs.657724	NM_003265	TLR3	1,307	-0,135	-0,220
Hs.174312	NM_138554	TLR4	-0,674	-0,245	-3,420
Hs.604542	NM_003268	TLR5	-1,858	0,235	0,100
Hs.662185	NM_006068	TLR6	3,363	0,015	0,670
Hs.659215	NM_016562	TLR7	-1,051	-2,025	-0,730
Hs.660543	NM_138636	TLR8	NA	NA	NA
Hs.87968	NM_017442	TLR9	NA	3,155	0,610
Hs.241570	NM_000594	TNF	-1,433	-1,515	-1,800
Hs.279594	NM_001065	TNFRSF1A	-0,639	0,735	1,910
Hs.368527	NM_019009	TOLLIP	1,662	0,575	0,820
Hs.591983	NM_004620	TRAF6	-1,086	-0,925	-1,100
Hs.524630	NM_003348	UBE2N	-0,803	-0,665	-0,600

6. Primers for reverse transcription quantitative PCR (RT-qPCR)

Table S4. Table of primers for conformational analysis

Gene name	Forward primer sequence	Reverse primer sequence
CCL20	TTTGCTCCTGGCTGCTTTGA	AGCAGTCAAAGTTGCTTGCTTC
CXCL10	CCTGCAAGCCAATTTTGTCCA	TGTGTGGTCCATCCTTGGAA
hβDEF 2	TGGTGAAGCTCCCAGCCATC	ACATGTCGCACGTCTCTGAT
IFNβ	ATGACCAACAAGTGTCTCCT	CTGTCCTTGAGGCAGTATTC
IL10	GACTTTAAGGGTTACCTGGGTTG	TCACATGCGCCTTGATGTCTG
IL1β	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
IL8	GAACTGAGAGTGATTGAGAGTGGA	CTCTTCAAAAACTTCTCCACAACC
RIG-I	ATGGAGGCTGCCACACTTTT	GCCATCATCCCCTTAGTAGAGC
TLR10	ACCGTGTCAACGAATCATCCA	ATGAGCTCAAAACCCCCACG
TLR1	AGTGTTTTCAAATTCAACCAGGA	AATGACAGGTCCAAGTGCTT
TLR2	GAGTTCTCCCAGTGTTTGGTGT	ACCTATCACTAGCAGGCAGG
TLR3	GTTGACTCAGGTACCCGATGA	ATACCTTGTGAAGTTGGCGG
TLR4	ATGCCAGGATGATGTCTGCC	GGGAGGTTGTCGGGGATTTT
TLR6	TCATGTTCCAAAAGACCTACCG	ACTCTGATAGAAAGCTCATGTCA
TNFα	GGCGTGGAGCTGAGAGATAAC	GGTGTGGGTGAGGAGCACAT
TOP1	CGCGCTCGTCCCTCC	AAATCCGCTTCGATCTGGGA
βACTIN	GGGACCTGACTGACTACCTC	AGCTTCTCCTTAATGTCACGC
GAPDH	GTCAGTGGTGGACCTGACCT	AGGGGTCTACATGGCAACTG