Supplementary Figure S3

- ***Western blot results for 12 proteins.*** Each of the following Western blots in Figures S3,A-N include choroid control samples C01, C02, C03, C04, C08, C09, C11, C13, metastasizing pUM samples UM91, UM93, UM94, UM56, UM62, UM67, UM85, UM86, and non-metastasizing pUM samples UM33, UM34, UM39, UM06, UM09, UM13, UM17, UM19. Blue control sample labels denote choroid tissue from eyes containing metastazising pUM; green labels denote choroid from non-metastasizing pUM. Red pUM sample labels denote specimens from Cleveland, OH; black labels denote samples from Liverpool, UK. Prior to blotting to PVDF membrane, sample amounts applied to SDS-PAGE (~10 µg) were equalized based on Coomassie blue staining intensities (see Supplementary Figure S2). The Chameleon Duo pre-stained protein ladder from LI-COR, USA was used to estimate molecular weight. Fluorescence intensity was measured with a LI-COR Odyssey CLx imaging system on the indicated bands using Image Studio 5.2. Intensity measurements and calculations are presented without adjustments for background or outlier values.

Figure S3A. Fluorescence immunoblot reactivity for glutathione S-transferase Omega-1, UniProt accession number P78471, molecular weight 27,566 Da. The blot was probed overnight at 4°C with anti-glutathione S-transferase Omega 1 (mouse mAb) at 0.5 µg/mL, #MABN642, EMD Millipore). Secondary antibody was donkey anti-mouse from LI-COR, USA (#925-68072) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

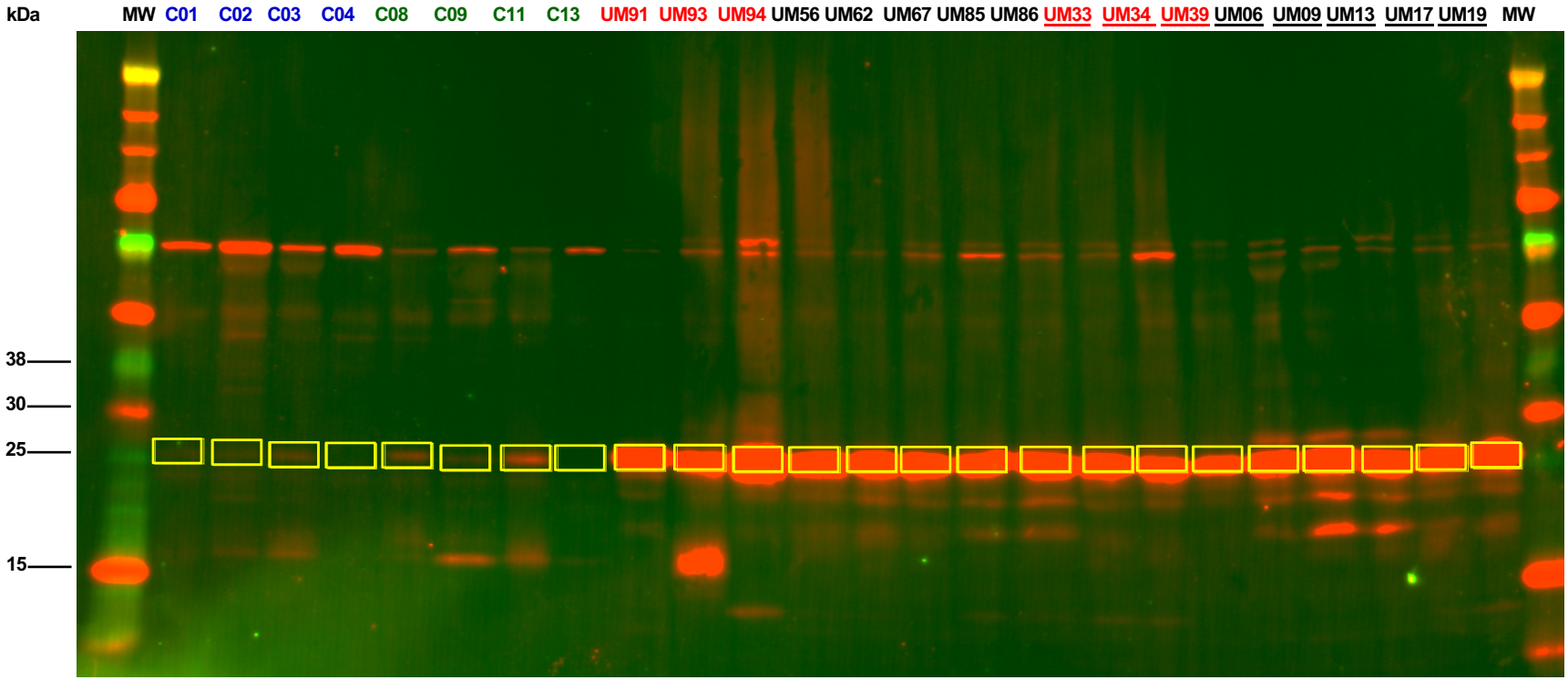

Figure S3A. Quantitation																								
Glutathione S-tansferase Omega-1	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	1318	1357	1393	1224	1421	1287	1764	1133	20034	8406	30562	14072	14866	16734	17964	25003	15606	13011	8252	31118	32130	27001	17171	19923
Average intensity ± RSD	1362 ± 14%								18455 ± 37%								20526 ± 42%							
Average pUM intensity / Control ± RSD									13.6 ± 37% iTRAQ=2.4								15.1 ± 42% iTRAQ=2.2							
Ave pUM Met / Ave pUM NoMet									0.9 iTRAQ=1.1															

Figure S3B. Fluorescence immunoblot reactivity for macrophage migration inhibitory factor, UniProt accession number P14174, molecular weight 12,476 Da. The blot was probed overnight at 4°C with anti-macrophage migration inhibitory factor (mouse mAb at 1.6 µg/mL, #MAB289-100, R&D Systems). Secondary antibody was donkey anti-mouse from LI-COR, USA (#925-68072) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

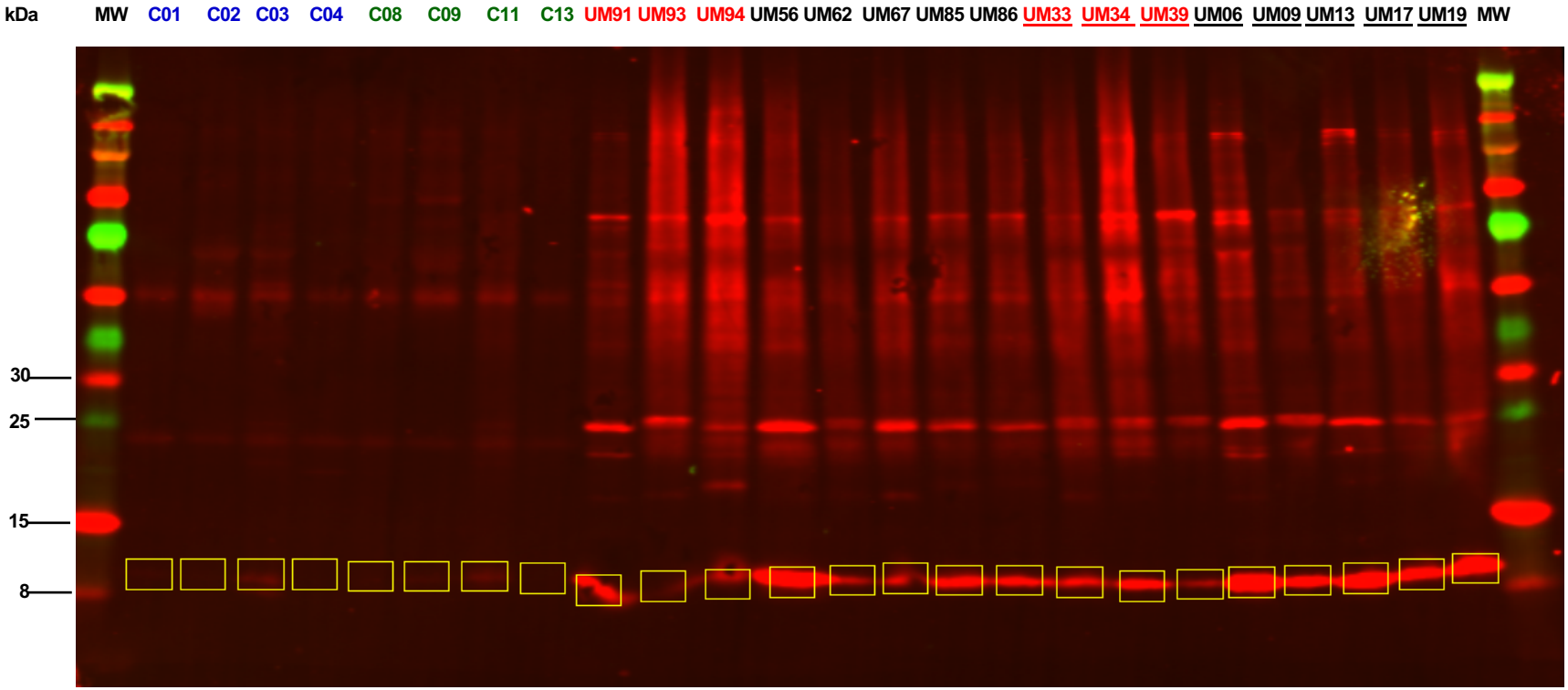

Figure S3B. Quantitation																								
Macrophage migration inhibitory factor	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	490	487	563	478	491	504	571	473	1116	543	731	2206	720	755	1049	911	851	1061	619	4202	1310	2697	1359	1996
Average intensity ± RSD	507 ± 8%								1004 ± 52%								1762 ± 67%							
Average pUM intensity / Control ± RSD									2.0 ± 52% iTRAQ=1.8								3.5 ± 67% iTRAQ=2.1							
Ave pUM Met / Ave pUM NoMet									0.6 iTRAQ=0.8															

Figure S3C. Fluorescence immunoblot reactivity for Syntenin-1, UniProt accession number O00560, molecular weight 32,424 Da. The blot was probed overnight at 4°C with anti-syntenin-1 (rabbit pAb at 1:1000 dilution, #A5360, AB clonal). Secondary antibody was goat anti-rabbit IgG from LI-COR, USA (#925-6807) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

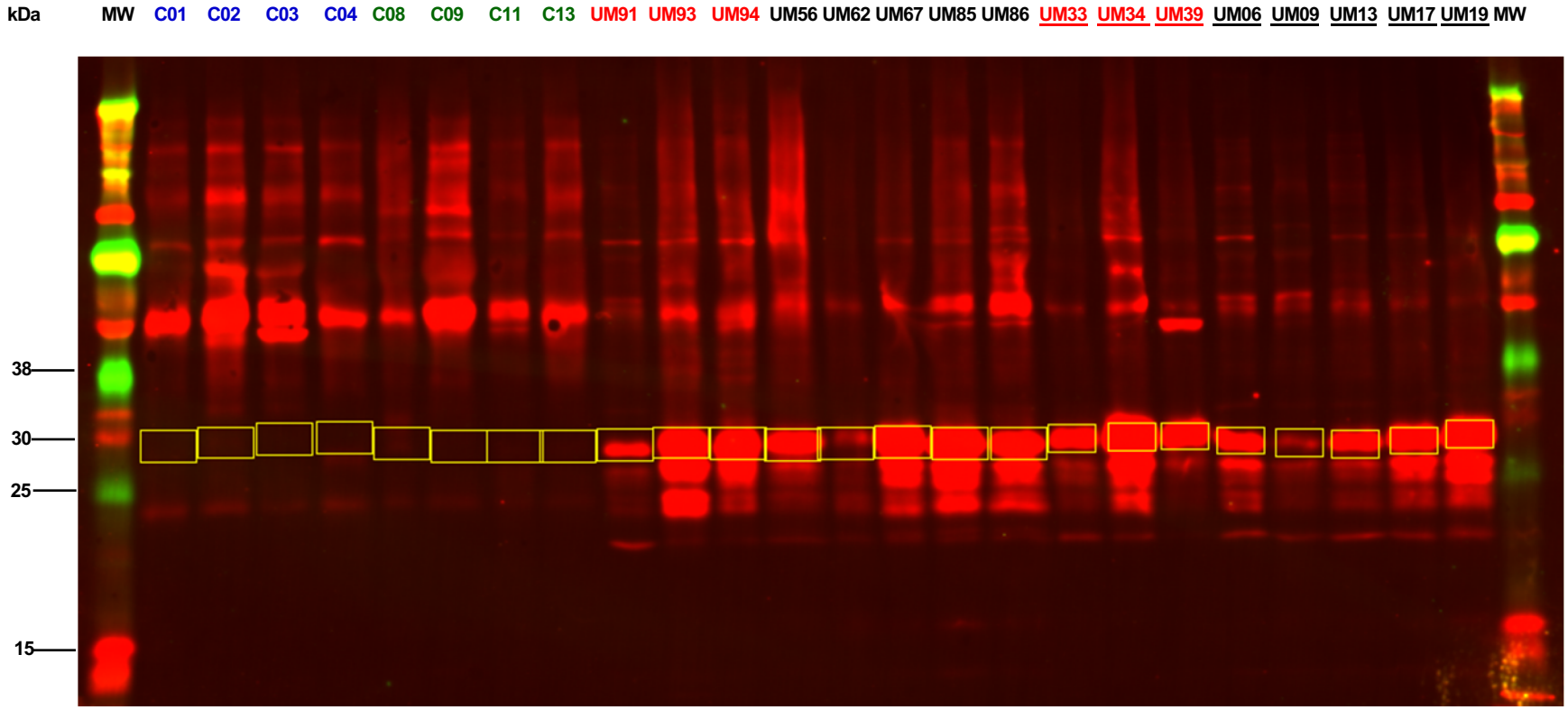

Figure S3C. Quantitation																								
Syntenin-1	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	2290	2453	2203	2246	2432	2267	2290	2335	6568	31723	32993	15595	4992	38751	57420	32464	10139	111403	16330	11923	3241	10293	18736	42560
Average intensity ± RSD	2315 ± 4%								25763 ± 64%								29078 ± 127%							
Average pUM intensity / Control ± RSD									11.9 ± 64% iTRAQ=1.6								12.1 ± 127% iTRAQ=1.2							
Ave pUM Met / Ave pUM NoMet									1.0 iTRAQ=1.3															

Figure S3D. Fluorescence immunoblot reactivity for G-protein coupled receptor 143, UniProt accession number P51810, molecular weight 43,878 Da. The blot was probed overnight at 4°C with anti-G-protein coupled receptor 143 (rabbit pAb at 1:2000, #A10568, ABclonal). Secondary antibody was goat anti-rabbit IgG from LI-COR, USA (#925-6807) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

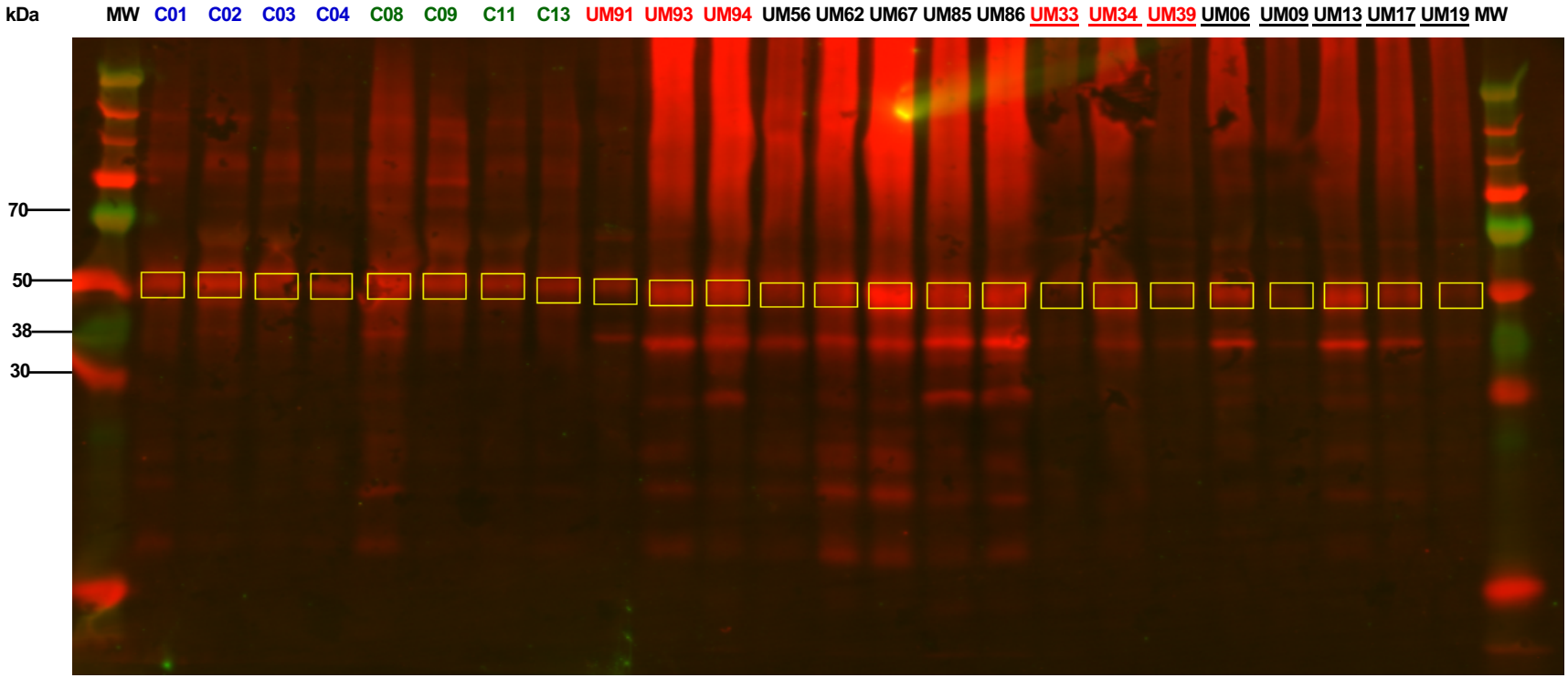

Figure S3D. Quantitation																								
G-Potein coupled receptor 143	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	6741	8950	5907	4513	9782	6903	5525	4706	3642	8870	9409	4957	9010	26354	10412	11149	2648	7116	3339	6111	2904	8924	5113	3031
Average intensity ± RSD	6628 ± 29%								10475 ± 66%								4898 ± 47%							
Average pUM intensity / Control ± RSD									1.6 ± 66% iTRAQ=1.9								0.7 ± 47% iTRAQ=1.2							
Ave pUM Met / Ave pUM NoMet									2.1 iTRAQ=1.5															

Figure S3E. Fluorescence immunoblot reactivity for HLA class II histocompatibility antigen DR alpha, UniProt accession number P01903, molecular weight 28,607 Da. The blot was probed overnight at 4°C with anti-HLA class II histocompatibility antigen, DR alpha (rabbit pAb at 1:2000, #A11787, ABclonal). Secondary antibody was goat anti-rabbit IgG from LI-COR, USA (#925-6807) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

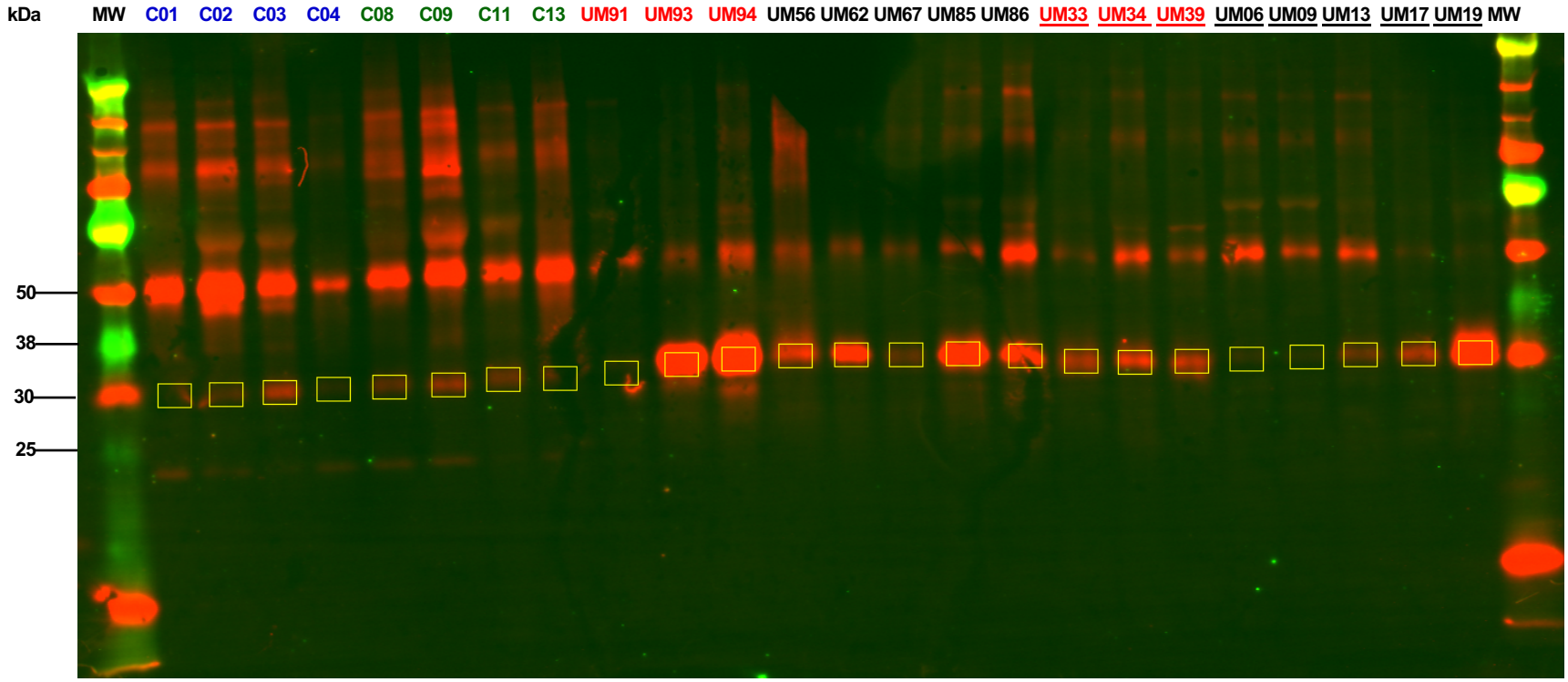

Figure S3E. Quantitation																								
HLA Class II histocompatibility antigen DR al	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	1346	1487	2077	1181	1545	1909	1682	1204	1284	10184	20579	2742	3725	1633	5778	3445	2317	2607	2380	1252	1200	1708	2219	6822
Average intensity ± RSD	1554 ± 21%								6171 ± 105%								2563 ± 70%							
Average pUM intensity / Control ± RSD									4.09 ± 105% iTRAQ=1.5								1.6 ± 58% iTRAQ=0.9							
Ave pUM Met / Ave pUM NoMet									2.4 iTRAQ=1.7															

Figure S3F. Fluorescence immunoblot reactivity for galectin-3 binding protein, UniProt accession number Q08380, molecular weight 65,331 Da. The blot was probed overnight at 4°C with anti-galectin-3 binding protein (goat pAb at 0.2 µg/mL, #AF2226, R&D Systems). Secondary antibody was donkey anti-goat from LI-COR, USA (#925-68074) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

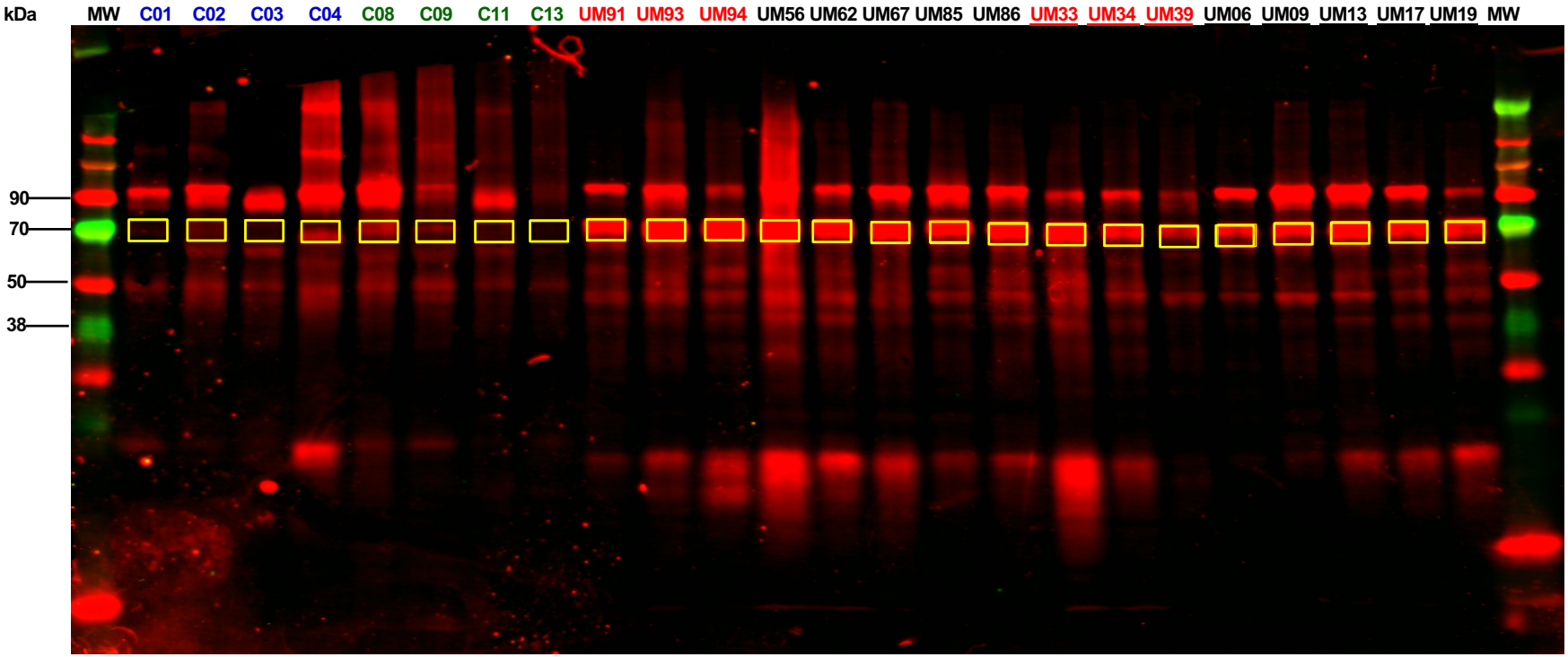

Figure S3F. Quantitation																								
Galectin-3 binding protein	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	1721	2180	1865	2832	2931	2205	2030	1704	6121	9236	9435	13416	6324	6387	6867	8142	6279	4111	3145	3912	4959	7837	4651	4877
Average intensity ± RSD	2184 ± 22%								8241 ± 30%								4971 ± 30%							
Average pUM intensity / Control ± RSD									3.8 ± 30% iTRAQ=1.1								2.3 ± 30% iTRAQ=0.9							
Ave pUM Met / Ave pUM NoMet									1.7 iTRAQ=1.3															

Figure S3G. Fluorescence immunoblot reactivity for vitronectin, UniProt accession number P04004, molecular weight 54,306 Da. The blot was probed overnight at 4°C with anti-vitronectin (mouse mAb at 0.4 µg/mL, #MAB2349, R&D Systems). Secondary antibody was donkey anti-mouse from LI-COR, USA (#925-68072) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

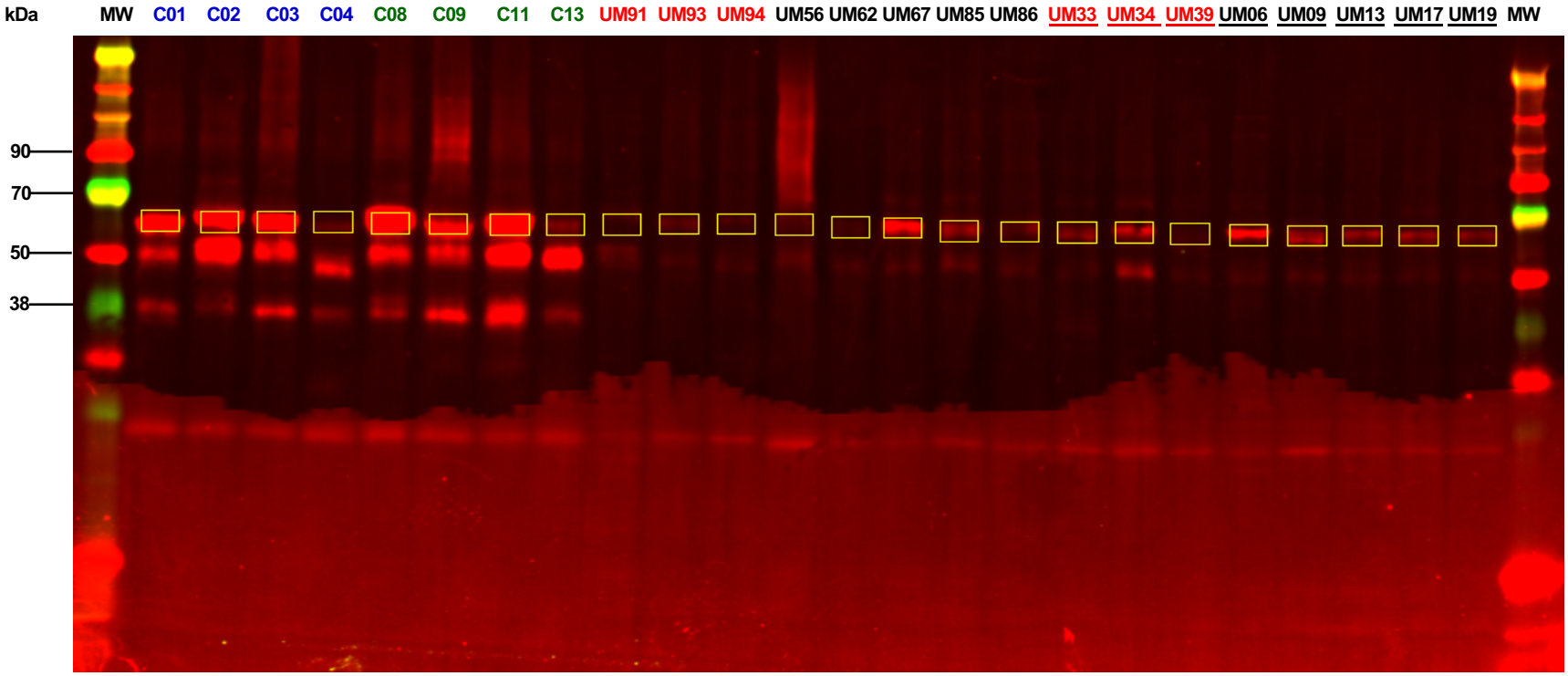

Figure S3G. Quantitation.																								
Vitronectin	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	3630	5617	5906	1219	4903	2597	6777	1401	1103	1257	1137	1230	1122	2282	1565	1266	1400	1661	1116	2070	1595	1439	1452	1351
Average intensity ± RSD	4006 ± 53%								1370 ± 29%								1511 ± 18%							
Average pUM intensity / Control ± RSD									0.3 ± 29% iTRAQ=0.3								0.4 ± 18% iTRAQ=0.3							
Ave pUM Met / Ave pUM NoMet									0.9 iTRAQ=1.0															

Figure S3H. Fluorescence immunoblot reactivity for Nidogen-2, UniProt accession number Q14112, molecular weight 151,254 Da. The blot was probed overnight at 4°C with anti-nidogen-2 (goat pAb at 0.3 µg/mL, #AF3385, R&D Systems). Secondary antibody was donkey anti-goat from LI-COR, USA (#925-68074) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

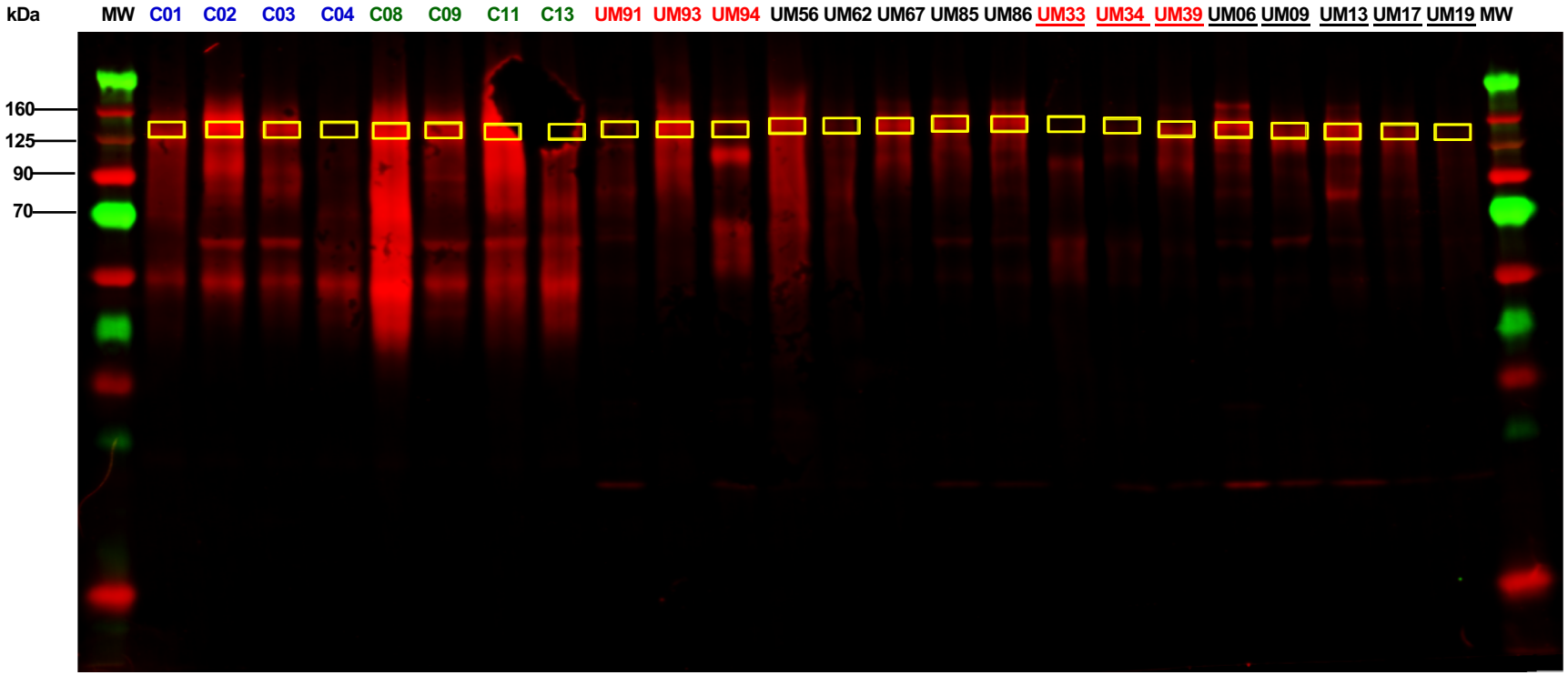

Figure S3H. Quantitation.																								
Nidogen-2	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	3177	13715	4854	991	10283	4452	18177	1199	1386	6303	1688	4108	1424	4083	3468	4277	833	1140	2585	5005	2544	4342	2321	1041
Average intensity ± RSD	7106 ± 88%								3342 ± 52%								2476 ± 62%							
Average pUM intensity / Control ± RSD									0.5 ± 52% iTRAQ=0.5								0.3 ± 62% iTRAQ=0.4							
Ave pUM Met / Ave pUM NoMet									1.3 iTRAQ=1.3															

Figure S3I. Fluorescence immunoblot reactivity for aspartate aminotransferase, UniProt accession number P00505, molecular weight 47,518 Da. The blot was probed overnight at 4°C with anti-aspartate aminotransferase (rabbit pAb at 1:2000, #A6915, ABclonal). Secondary antibody was goat anti-rabbit IgG from LI-COR, USA (#925-6807) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

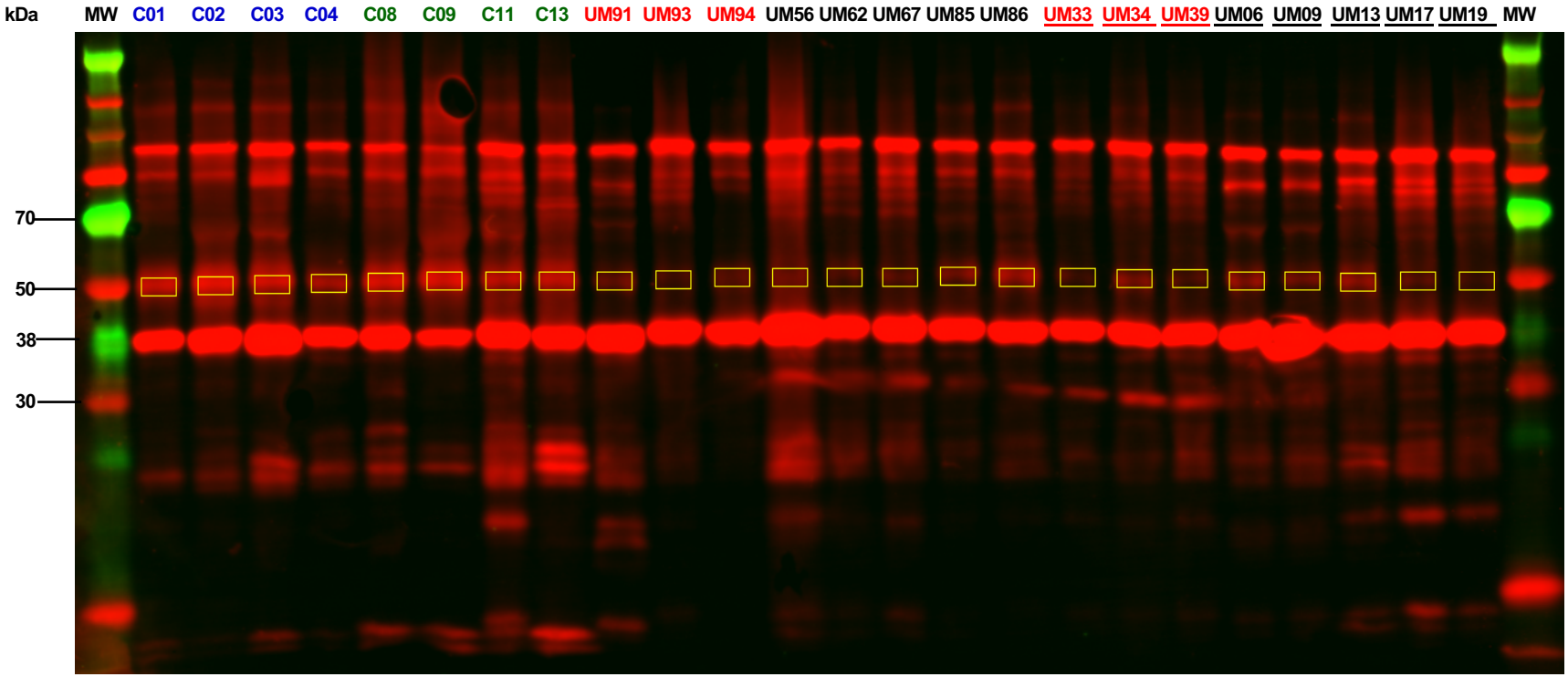

Figure S3I. Quantitation																								
Aspartate aminotransferase	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	4333	5931	4605	3059	5196	5485	3992	4214	1820	1394	1709	2092	1490	1635	2042	3080	1334	2162	1484	2434	1865	2554	1632	1330
Average intensity ± RSD	4602 ± 20%								1908 ± 28%								1849 ± 26%							
Average pUM intensity / Control ± RSD									0.4 ± 28% iTRAQ=0.5								0.4 ± 26% iTRAQ=0.6							
Ave pUM Met / Ave pUM NoMet									1.0 iTRAQ=0.8															

Figure S3J. Fluorescence immunoblot reactivity for lysosome membrane protein 2, UniProt accession number Q14108, molecular weight 54,290 Da. The blot was probed overnight at 4°C with anti-lysosome membrane protein 2 (goat pAb at 0.2 µg/mL, #AF1966, R&D Systems). Secondary antibody was donkey anti-goat from LI-COR, USA (#925-68074) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

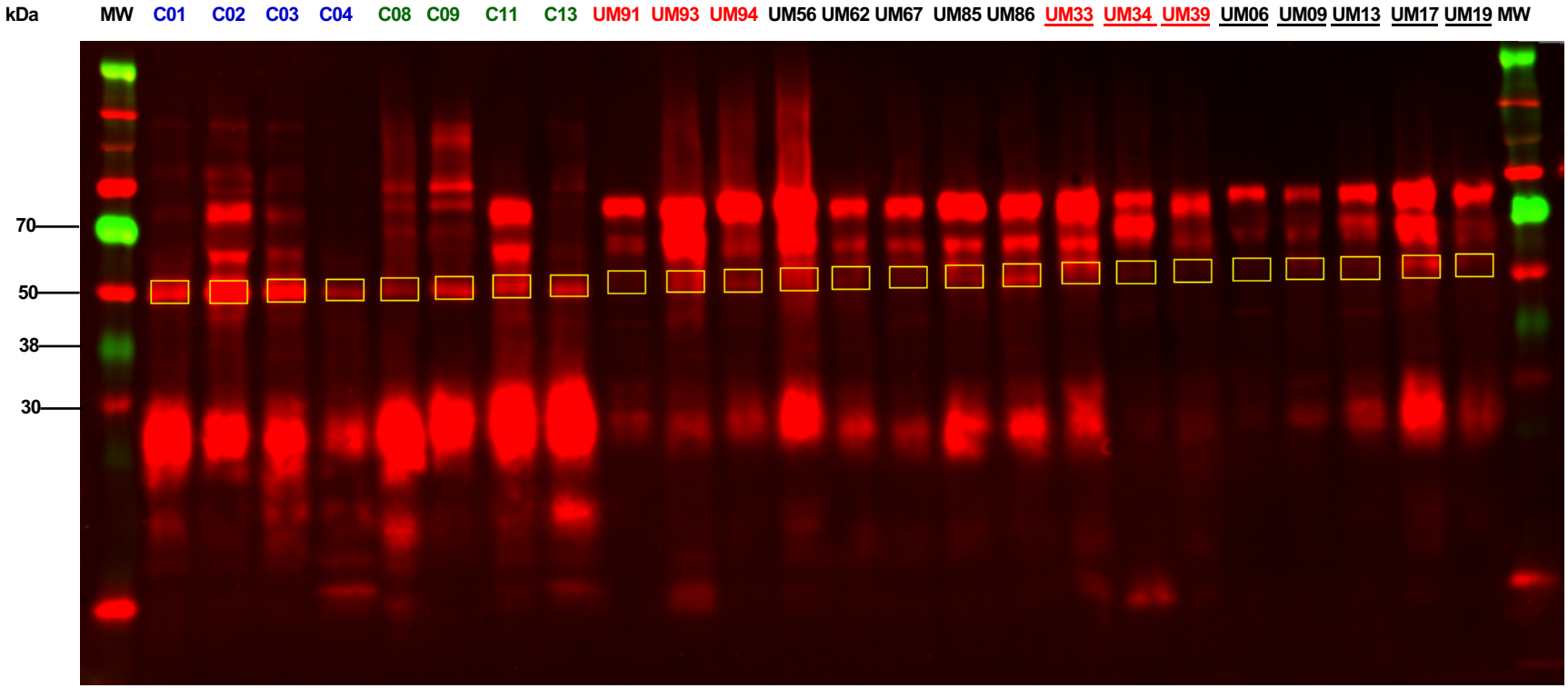

Figure S3J. Quantitation																								
Lysosome membrane protein 2	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	3597	5836	4252	2131	2125	2886	3087	2681	1431	2923	2152	3044	1796	1674	2418	2835	2729	1419	1236	1101	1496	1583	2778	1569
Average intensity ± RSD	3324 ± 37%								2284 ± 27%								1739 ± 37%							
Average pUM intensity / Control ± RSD									0.7 ± 27% iTRAQ=0.8								0.5 ± 37% iTRAQ=0.7							
Ave pUM Met / Ave pUM NoMet									1.3 iTRAQ=1.2															

Figure S3K. Fluorescence immunoblot reactivity for lactoylglutathione lyase, UniProt accession number Q04760, molecular weight 20,778 Da. The blot was probed overnight at 4°C with anti-lactoylglutathione lyase (rabbit pAb at 1:800, #A1932, ABclonal). Secondary antibody was goat anti-rabbit IgG from LI-COR, USA (#925-6807) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

Figure S3K. Quantitation																								
Lactoylglutathione lyase	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	1622	1230	13587	1385	1645	719	3378	1362	12370	10417	3868	11816	4280	3186	3889	6563	2601	14444	5689	5825	10490	13348	2749	1520
Average intensity ± RSD	3116 ± 138%								7049 ± 55%								7083 ± 71%							
Average pUM intensity / Control ± RSD									2.3 ± 55% iTRAQ=0.8								2.3 ± 71% iTRAQ=1.0							
Ave pUM Met / Ave pUM NoMet									1.0 iTRAQ=0.8															

Figure S3L. Fluorescence immunoblot reactivity for cAMP-dependent protein kinase type II-alpha regulatory subunit, UniProt accession number P13861, molecular weight 45,518 Da. The blot was probed overnight at 4°C with anti-cAMP-dependent protein kinase type II-alpha regulatory subunit (rabbit mAb at 0.6 µg/mL, # MAB8000, R&D Systems). Secondary antibody was goat anti-rabbit IgG from LI-COR, USA (#925-6807) used at a dilution of 1:5000, 2-3 h, room temperature in the dark.

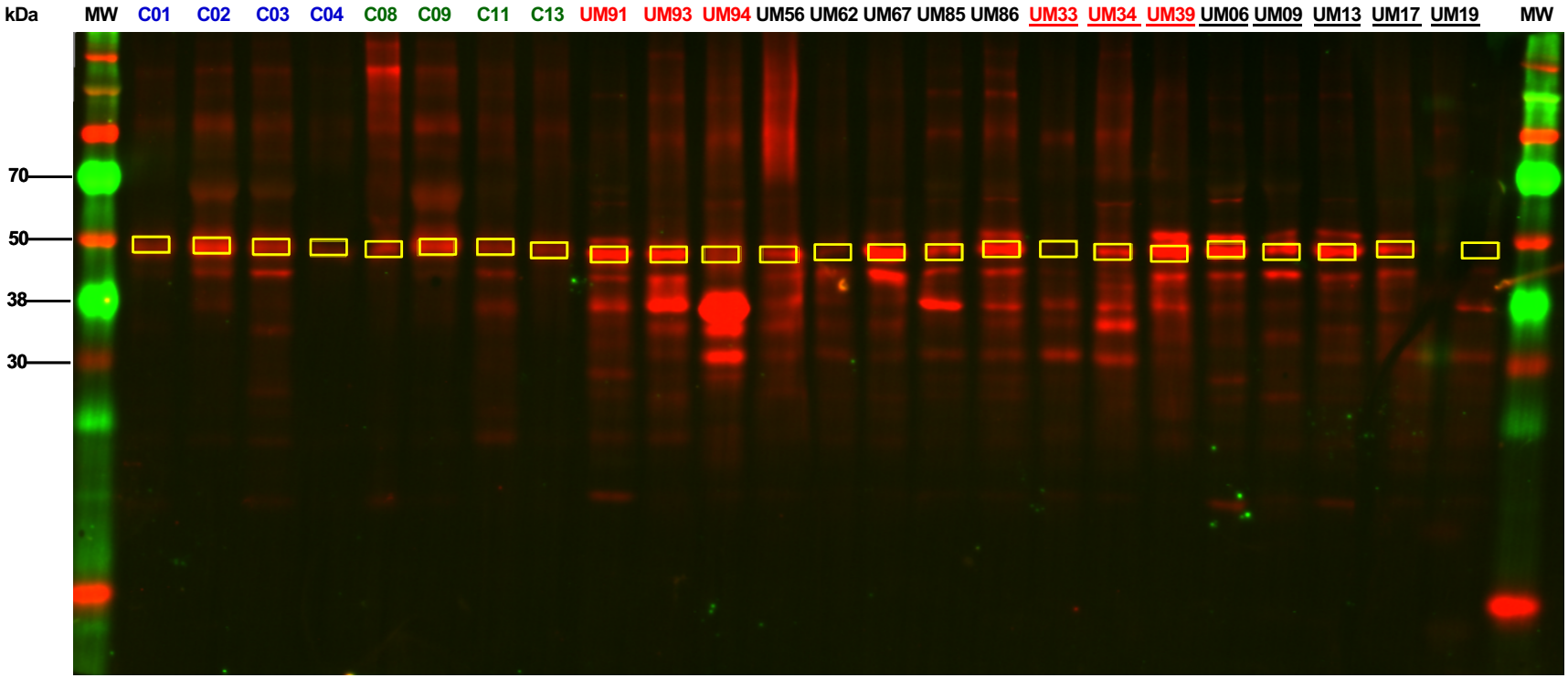

Figure S3L. Quantitation																									
cAMP-Dependent protein kinase type II-alp	C01	C02	C03	C04	C08	C09	C11	C13	UM91	UM93	UM94	UM56	UM62	UM67	UM85	UM86	UM33	UM34	UM39	UM06	UM09	UM13	UM17	UM19
Fluorescence intensity	1624	2800	2095	1198	1644	2506	1627	1633	3401	3097	2040	2239	1175	4421	2074	3454	890	2176	4494	4094	2234	2982	2009	584
Average intensity ± RSD	1891 ± 28%								2738 ± 38%								2433 ± 57%							
Average pUM intensity / Control ± RSD									1.4 ± 38% iTRAQ=0.6								1.3 ± 57% iTRAQ=0.8							
Ave pUM Met / Ave pUM NoMet									1.1 iTRAQ=0.8															