Online -only supplemental material

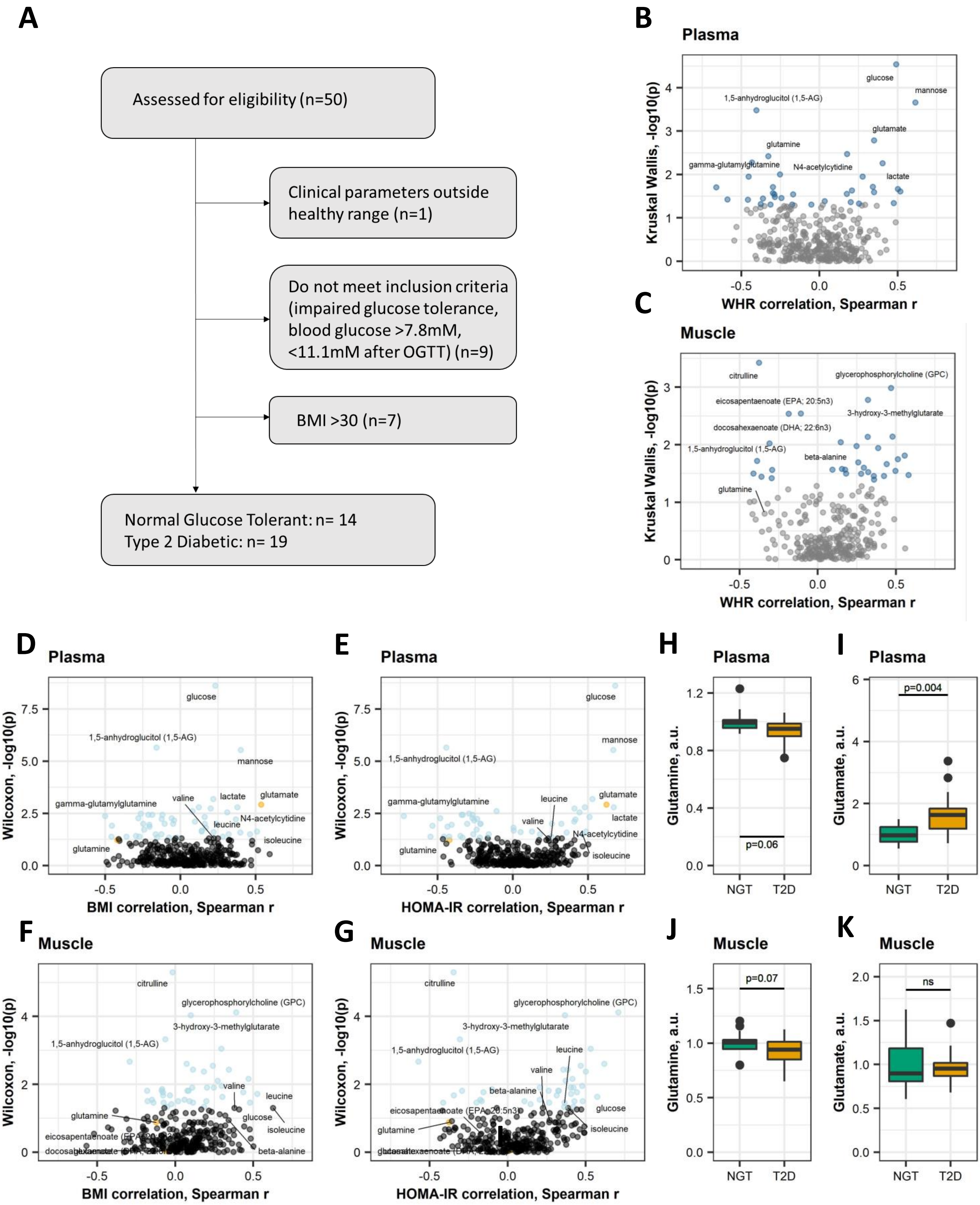
Glutamine Regulates Skeletal Muscle Immunometabolism in Type 2 Diabetes

Lucile Dollet[1], Michael Kuefner[1], Elena Caria[1], David Rizo-Roca[2], Logan Pendergrast[2], Ahmed M. Abdelmoez[2], Håkan KR Karlsson[2], Emilie Dalbram[3], Jonas Treebak[3], Jun Harada[4], Erik Näslund[5], Mikael Rydén[6], Juleen R. Zierath[1,2], Nicolas J. Pillon[1], Anna Krook[1].

[1]Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institutet, 171 77 Stockholm, Sweden.[2]Department of Molecular Medicine and Surgery, Integrative, Physiology, Karolinska Institutet, 171 77, Stockholm, Sweden. [3]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [4]Cardiovascular-Metabolics Research Laboratories, Daiichi Sankyo Co., Ltd., Tokyo, Japan. [5]Division of Surgery, Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, 182 88 Stockholm, Sweden. [6]Department of Medicine, Huddinge, Karolinska Institutet Stockholm Sweden

Mouse gene	Ensembl reference	Human gene	Ensembl ref	mouse_logFC	mouse_P.Value	human_logFC	human_P.Value
Defa22	ENSMUSG00000074443	DEFA5	ENSG00000164816	0.092528	0.027769	0.140774	0.01513
Defa28	ENSMUSG00000074434	DEFA5	ENSG00000164816	-0.15117	0.016915	0.140774	0.01513
Defa40	ENSMUSG00000074441	DEFA5	ENSG00000164816	0.084919	0.044609	0.140774	0.01513
Fndc1	ENSMUSG00000071984	FNDC1	ENSG00000164694	-0.17304	0.011134	-0.16488	0.022891
Grb10	ENSMUSG00000020176	GRB10	ENSG00000106070	-0.14901	0.018904	-0.15099	0.022287
Hba-a1	ENSMUSG00000069919	HBA1	ENSG00000206172	-1.03272	0.000178	-1.13577	0.000698
Hba-a1	ENSMUSG00000069919	HBA2	ENSG00000188536	-1.03272	0.000178	-1.27445	0.000917
Mrgprb2	ENSMUSG00000050425	MRGPRX4	ENSG00000179817	0.112245	0.036967	0.217329	0.048118
Ptx4	ENSMUSG00000044172	PTX4	ENSG00000251692	-0.22896	0.000259	-0.20994	0.018986
Rsad2	ENSMUSG00000020641	RSAD2	ENSG00000134321	-0.26204	0.00066	-0.12742	0.02321
Snca	ENSMUSG00000025889	SNCA	ENSG00000145335	-0.18844	0.005769	-0.17318	0.009612
Tas2r120	ENSMUSG00000059382	TAS2R31	ENSG00000256436	0.087108	0.016376	-0.19283	0.00415
Tas2r120	ENSMUSG00000059382	TAS2R19	ENSG00000212124	0.087108	0.016376	0.32795	0.005263
Zfp992	ENSMUSG00000070605	ZNF34	ENSG00000196378	-0.08283	0.032177	0.10602	0.048793

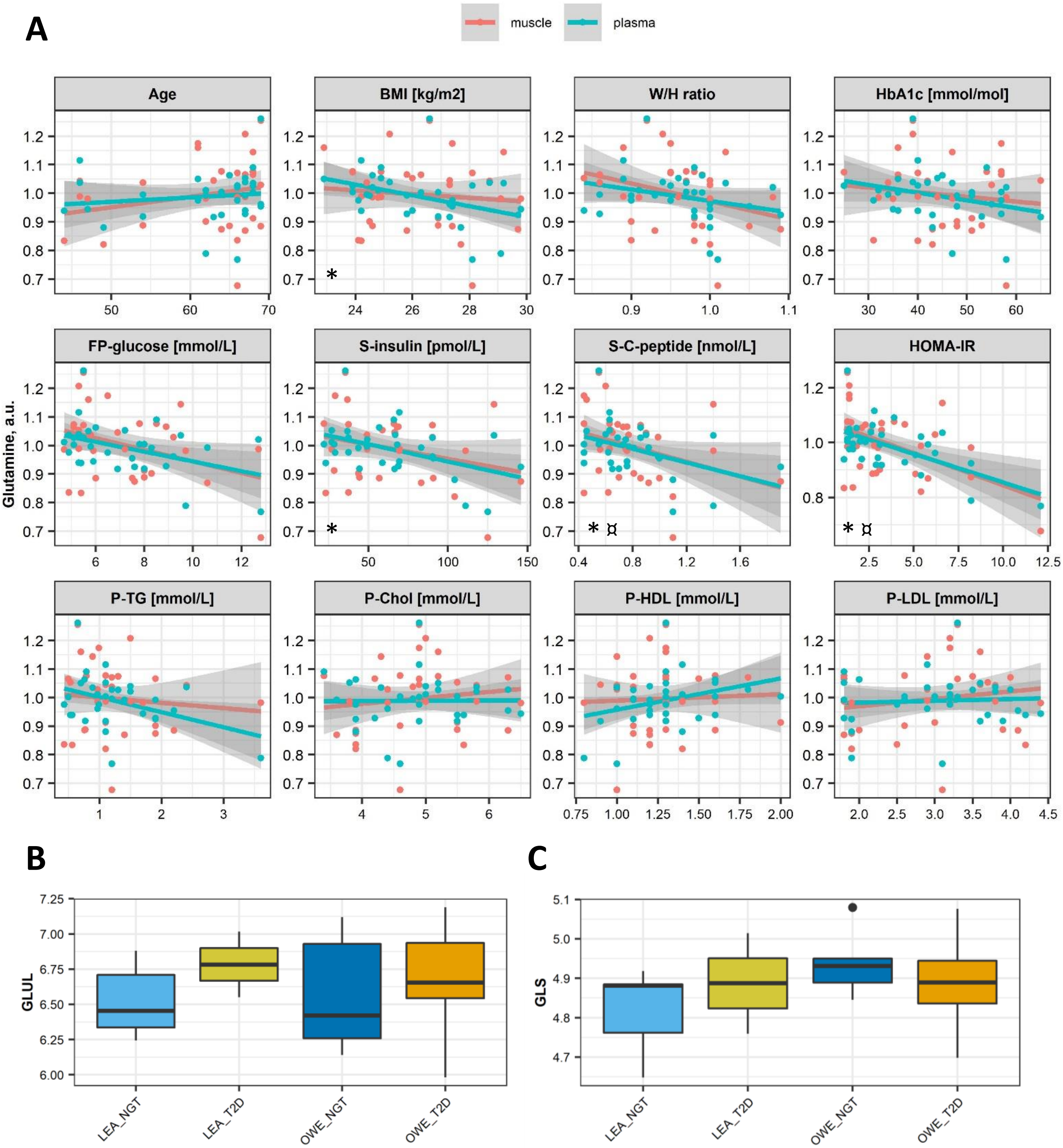
Supplemental Table 1: List of the genes commonly altered by glutamine administration in HFD fed mice ($p<0.05$) and altered in skeletal muscle of humans with high or low plasma glutamine levels ($p<0.05$). Log fold-change and adjusted p-value are indicated for both mice and human dataset.



Supplemental figure 1

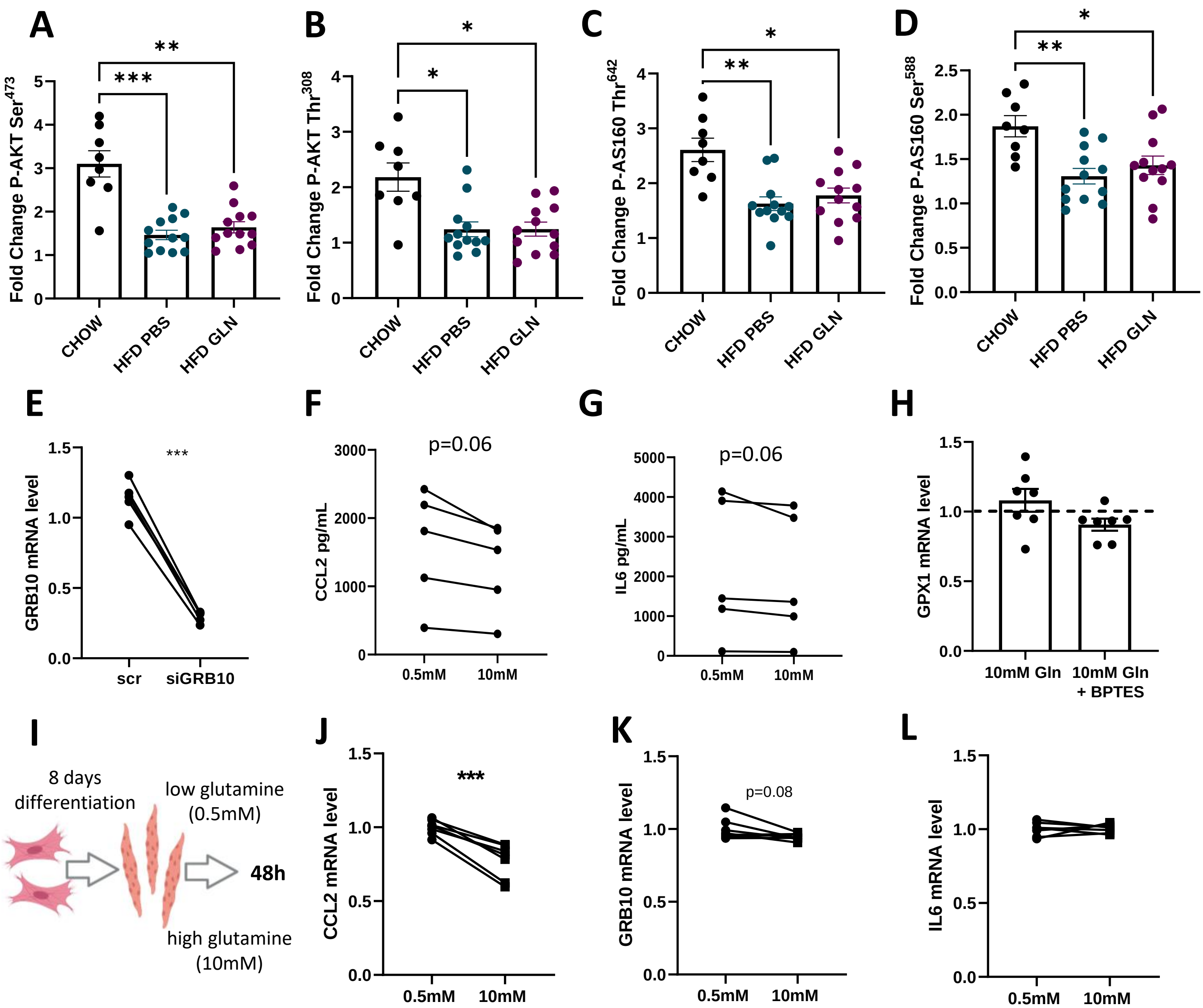
Supplemental Figure legends:

Supplemental Figure 1: Details of the cohort recruitment and criteria for inclusion/exclusion (A). Volcano plots of metabolites in plasma (B) and skeletal muscle (C) correlating with waist/hip ratio (WHR). Spearman correlation and Wilcoxon comparison across groups. Blue color indicates significance at $p<0.05$. Volcano plots of metabolites in plasma and skeletal muscle correlating with BMI and HOMA-IR (D-G). Spearman correlation and Wilcoxon comparison between NGT (lean+overweight) and T2D (lean+overweight) groups. Blue color indicates significance at $p<0.05$ (D-G). Plasma glutamine and glutamate levels (H-I) and skeletal muscle glutamine and glutamate levels (J-K). Mann-Whitney test between NGT and T2D groups.



Supplemental figure 2

Supplemental Figure 2: Correlations between clinical parameters and plasma (blue) and muscle (red) glutamine level. *p<0.05 for plasma, ¤p<0.05 for muscle (A). *GLUL* (B) and *GLS* (C) gene expression level in human skeletal muscle. n= 27.



Supplemental figure 3

Supplemental Figure 3:

Fold-change in Akt phosphorylation (P-AKT) and AS160 phosphorylation (P-AS160) in lysates of soleus skeletal muscle incubated in the absence or presence of a submaximal dose of insulin (0.36 nmol/L) for 20 min (A-D). Data are mean ± SEM. Kruskal-Wallis test with Dunn's multiple comparison test. *GRB10* mRNA expression level in myotubes 48h after transfection using either a scramble siRNA (scr) or a siRNA targeting GRB10 (siGRB10) (E). n=5. IL6 and CCL2 content in the medium collected after 24h from skeletal muscle cells differentiated during 8 days in low (0.5 mM) or high (10 mM) (F-G). n=5. BPTES was added for 24h and *GPX1* (H) mRNA level were measured. n=7. Statistical effect was measured by paired Student's *t*-test. *p<0.05; **p<0.01; ***p<0.001. Skeletal muscle cells were differentiated during 7 days in myotubes in classical 2mM glutamine medium, and then exposed to low (0.5 mM) or high (10 mM) concentration of glutamine during 48h (I), and *CCL2* (J), *GRB10* (K) and *IL6* (L) mRNA level were measured. N=7. Statistical effect was measured by paired Student's *t*-test. *p<0.05; **p<0.01; ***p<0.001.