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Supplemental Material to:

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MAPK14/p38α-dependent modulation of glucose metabolism affects ROS levels and autophagy during starvation

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Supplementary Figure 1

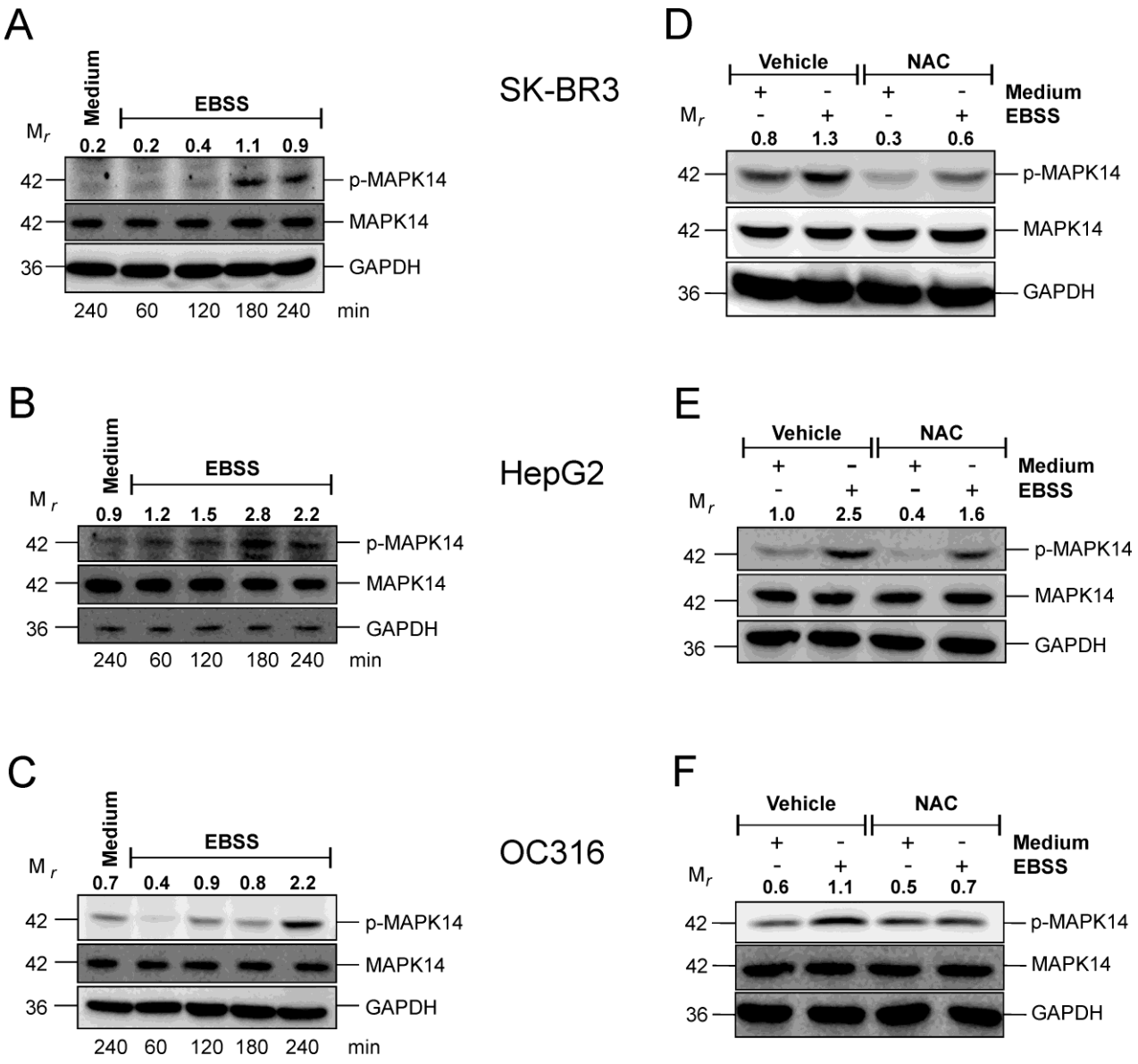

Figure S1. Starvation induces MAPK14 phospho-activation in cancer cell lines. SK-BR3 (**A**), HepG2 (**B**) and OC316 (**C**) cells were starved for the indicated times and western blot analyses of phospho-MAPK14 were performed. GAPDH was used as loading control. SK-BR3 (**D**), HepG2 (**E**) and OC316 (**F**) cells were pretreated for 1 h with either vehicle or 5 mM NAC and then starved for 3 h. Western blot analyses of phospho-MAPK14 were performed. GAPDH was used as loading control. Western blots are from 1 experiment representative of 3 that gave similar results. Numbers indicate relative phospho-MAPK14/ MAPK14 ratios.

Supplementary Figure 2

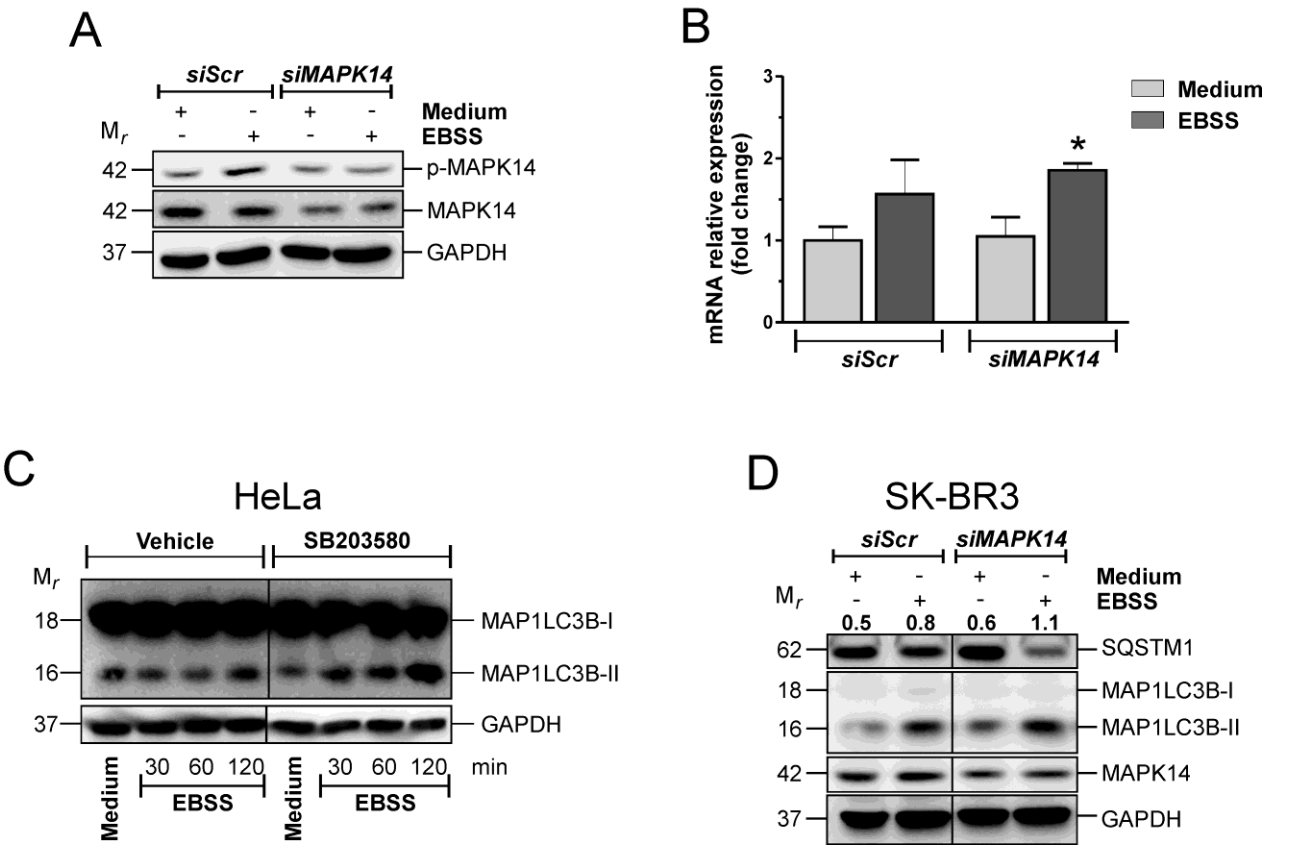

Figure S2. MAPK14 negatively regulates autophagy. (**A**) HeLa cells were transfected with either a nontargeting siRNA (*siScr*) or an siRNA targeting *MAPK14* (*siMAPK14*). Twelve h after transfection cells were starved for 3 h and western blot analysis of phospho-MAPK14 was performed. MAPK14 and GAPDH were used as knockdown and loading control, respectively. (**B**) HeLa cells were transfected and treated as in (**A**). The relative expressions of *SQSTM1* mRNAs were assayed by real-time PCR. Data are presented as mean ± SD of n = 3 independent experiments. **P*<0.05 against *siMAPK14* medium. (**C**) HeLa cells were pretreated for 1 h with either vehicle or 10 μM of the MAPK14 inhibitor SB203580 and then starved for the indicated times. Western blot analysis of MAP1LC3B was performed. GAPDH was used as loading control. (**D**) SK-BR3 cells were transfected with either a nontargeting siRNA (*siScr*) or an siRNA targeting *MAPK14* (*siMAPK14*). Twelve h after transfection cells were starved for 3 h and western blot analysis of MAP1LC3B and SQSTM1 was performed. MAPK14 and GAPDH were used as

knockdown and loading control, respectively. Numbers indicate relative MAP1LC3B-II/GAPDH ratios.

Supplementary Figure 3

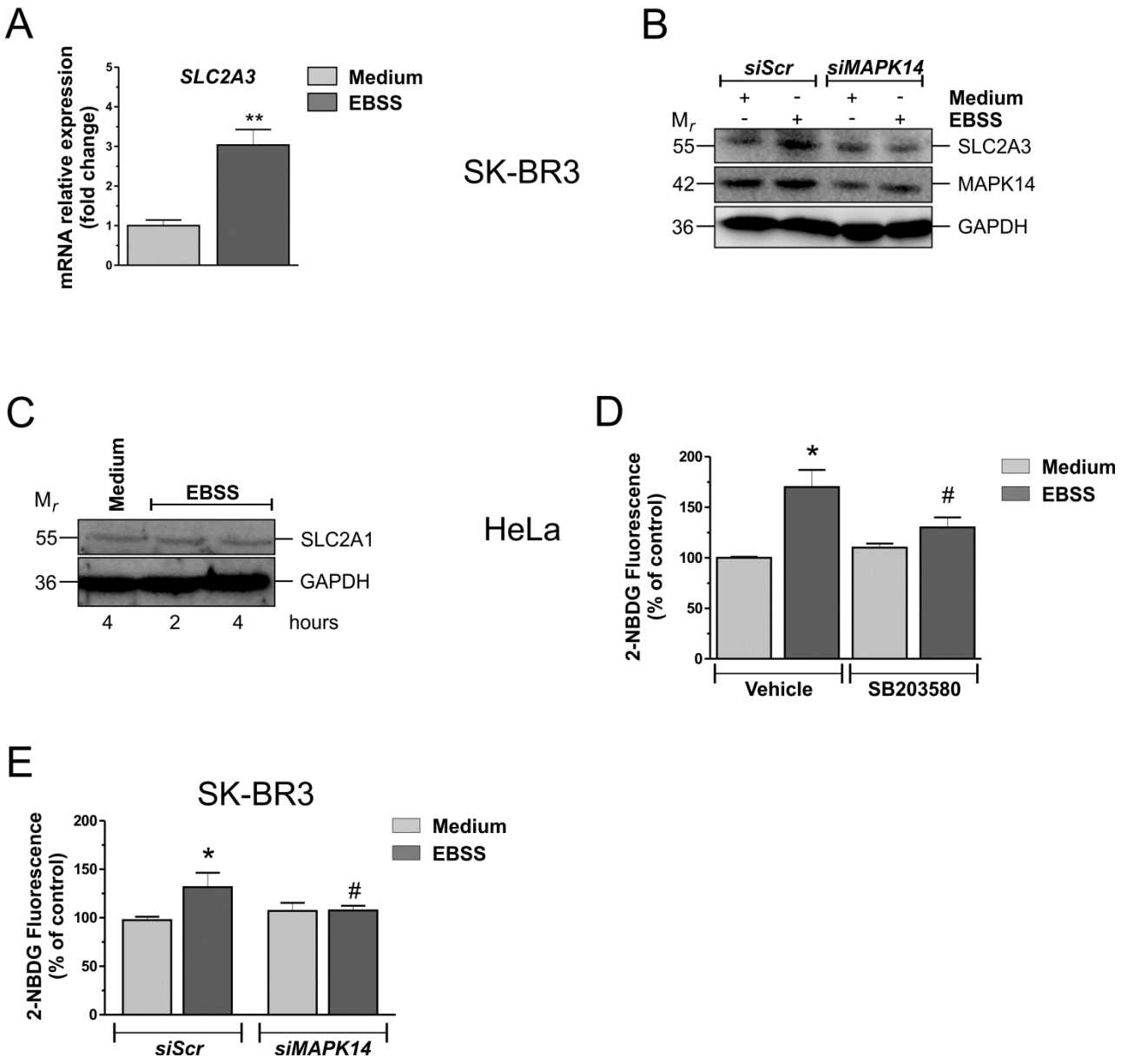

Figure S3. MAPK14 increases *SLC2A3* expression and glucose uptake. (**A**) SK-BR3 cells were starved for 3 h and the relative expression of *SLC2A3* mRNA was assayed by real-time PCR. Data are presented as mean ± SD of n = 3 independent experiments. ***P*<0.01 against Medium. (**B**) SK-BR3 cells were transfected with either a nontargeting siRNA (*siScr*) or an siRNA targeting *MAPK14* (*siMAPK14*). Twelve h after transfection cells were starved for the indicated times and western blot analysis of SLC2A3 was performed. MAPK14 and GAPDH were used as knockdown and loading control, respectively. Western blots are from 1 experiment representative of 3 that gave

similar results. (**C**) HeLa cells were starved for the indicated times and western blot analysis of SLC2A1 was performed. GAPDH was used as loading control. (**D**) HeLa cells were pretreated 1 h with either vehicle or 10 μM of the MAPK14 inhibitor SB203580 and then starved for 3 h. Thirty min before the end of the experimental time, cells were incubated with 100 μM of the glucose fluorescent analog 2-NBDG and cytofluorometrically analyzed. Data are presented as mean ± SD of n = 3 independent experiments. **P*<0.05 against vehicle medium. $^{\#}$*P*<0.05 against vehicle EBSS. (**E**) SK-BR3 cells were treated as in (**B**) and starved for 3 h. Thirty min before the end of the experimental time, cell were incubated with 100 μM of the glucose fluorescent analog 2-NBDG and cytofluorometrically analyzed. Data are presented as mean ± SD of n = 3 independent experiments. **P*<0.05 against siScr medium. $^{\#}$*P*<0.05 against siScr EBSS.

Supplementary Figure 4

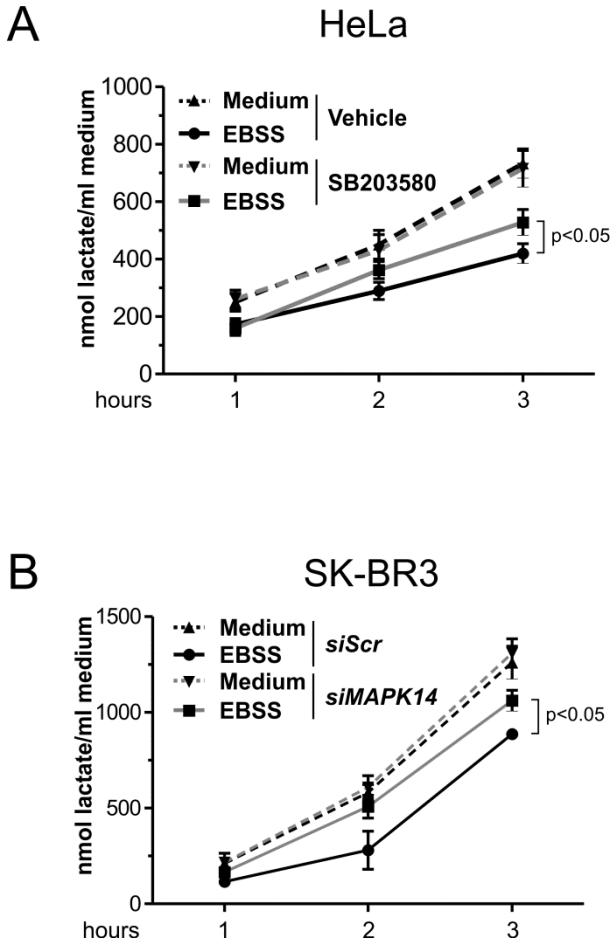

Figure S4. MAPK14 activation reduces extracellular lactate**.** (**A**) HeLa cells were pretreated for 1 h with either vehicle or 10 μM of the MAPK14 inhibitor SB203580, starved for the indicated times and extracellular lactate assayed spectrophotometrically. Data are presented as mean ± SD of n = 3 independent experiments. (**B**) SK-BR3 cells were transfected with either a non-targeting siRNA (*siScr*) or an siRNA targeting *MAPK14* (*siMAPK14*). Twelve h after transfection cells were starved for the indicated times and extracellular lactate assayed spectrophotometrically. Data are presented as mean ± SD of n = 3 independent experiments.

Supplementary Figure 5

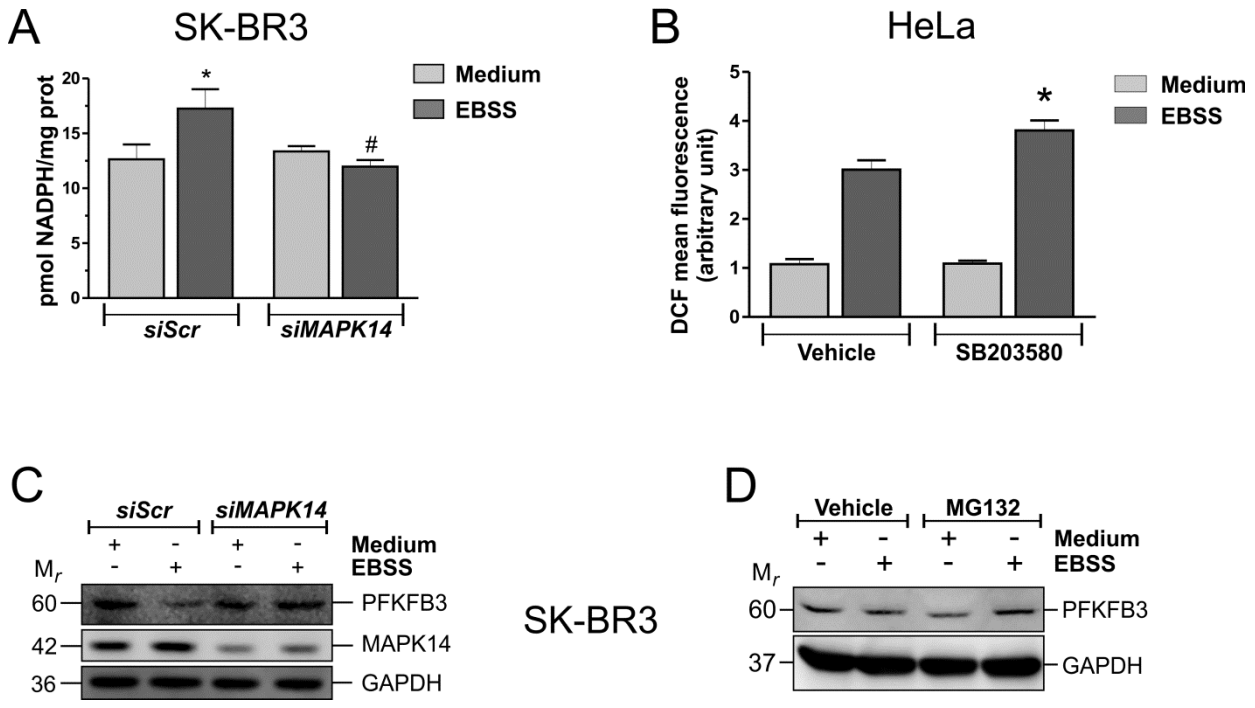

Figure S5. MAPK14 affects NADPH, ROS and PFKFB3 levels. (**A**) SK-BR3 cells were transfected with either a non-targeting siRNA (*siScr*) or an siRNA targeting *MAPK14* (*siMAPK14*). Twelve h after transfection cells were starved for 3 h and NADPH assayed spectrophotometrically. Data are presented as mean ± SD of n = 3 independent experiments. **P*<0.05 against siScr Medium. $^{\#}$*P*<0.05 against siScr EBSS. (**B**) HeLa cells were pretreated for 1 h with either vehicle or 10 μM of the MAPK14 inhibitor SB203580 (SB) and starved for 3 h. Thirty min before the end of the experimental time, cells were incubated with 50 μM H_2DCF-DA and ROS levels were measured cytofluorometrically. Data are presented as mean ± SD of n = 3 independent experiments. **P*<0.05 against siScr EBSS. (**C**) SK-BR3 cells were treated as in (**A**). Twelve h after transfection cells were starved for 3 h and western blot analysis of PFKFB3 was performed. MAPK14 and GAPDH were used as knockdown and loading control, respectively. (**D**) SK-BR3 cells were pretreated for 1 h with either vehicle or 10 μM of the proteasome inhibitor MG132 and then starved for 3 h. Western

blot analysis of PFKFB3 was performed. GAPDH was used as loading control. Western blots are from 1 experiment representative of 3 that gave similar results.