Supplementary Figure legends

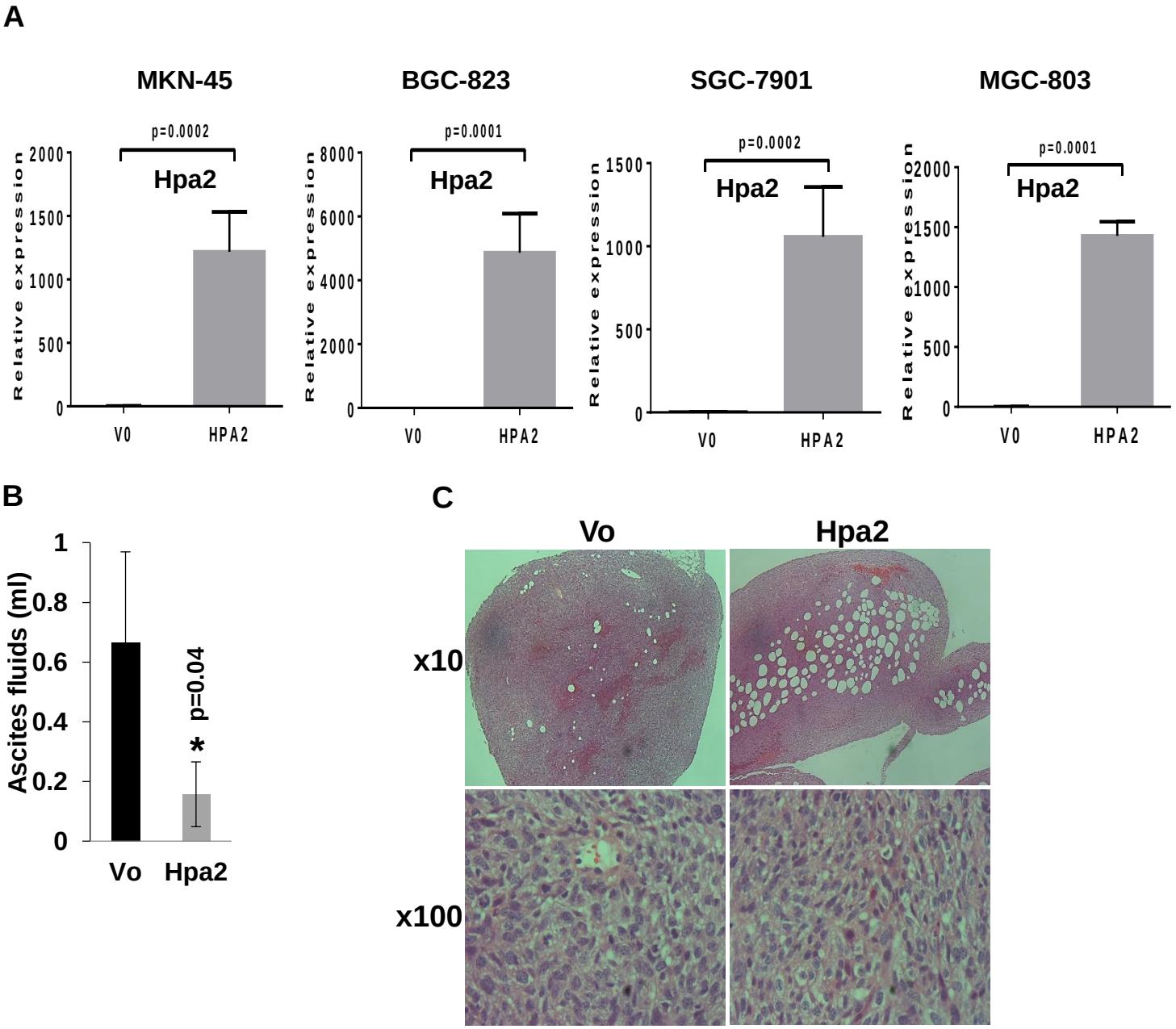
Suppl. Figure 1. **A.** Stably transfected gastric carcinoma cells. The indicated cell line was transfected with control empty plasmid (Vo) or Hpa2 gene construct. Following selection, cells were expanded and pooled; Total RNA was extracted and subjected to qPCR applying primers specific for Hpa2. Shown are Hpa2 expression levels in transfected cells vs control (Vo) cells, set arbitrarily to a value of 1. **B**. Decreased tumor burden by Hpa2 cells. Control (Vo) and Hpa2 overexpressing MGC-803 cells were implanted intra-peritoneal into NOD/SCID mice (n=7). After 14 days mice were sacrificed and ascites fluids were collected. Shown is the average volume (ml) of ascites fluids collected from mice implanted with control (Vo) and Hpa2 cells. Tumors were collected from the peritoneum, fixed in formalin, and processed for histological examination (**C**). Shown are images of H&E staining of control (Vo, left) and Hpa2 tumor sections (right) at low (upper panels) and high (lower panels) magnification.

Suppl. Fig. 2. Hpa2 expression is induced by MG132. Mouse embryonic fibroblasts (MEF) (**A**) and mouse Lewis lung carcinoma (**B**) cells were treated with MG132 (20 μM) for the time indicated (A) or the indicated concentration of MG132 for 16 hours (B). Total RNA was then extracted and subjected to qPCR applying primers specific for Hpa2 (upper panels), MIP2 (middle panels) and TNF-α (lower panels).

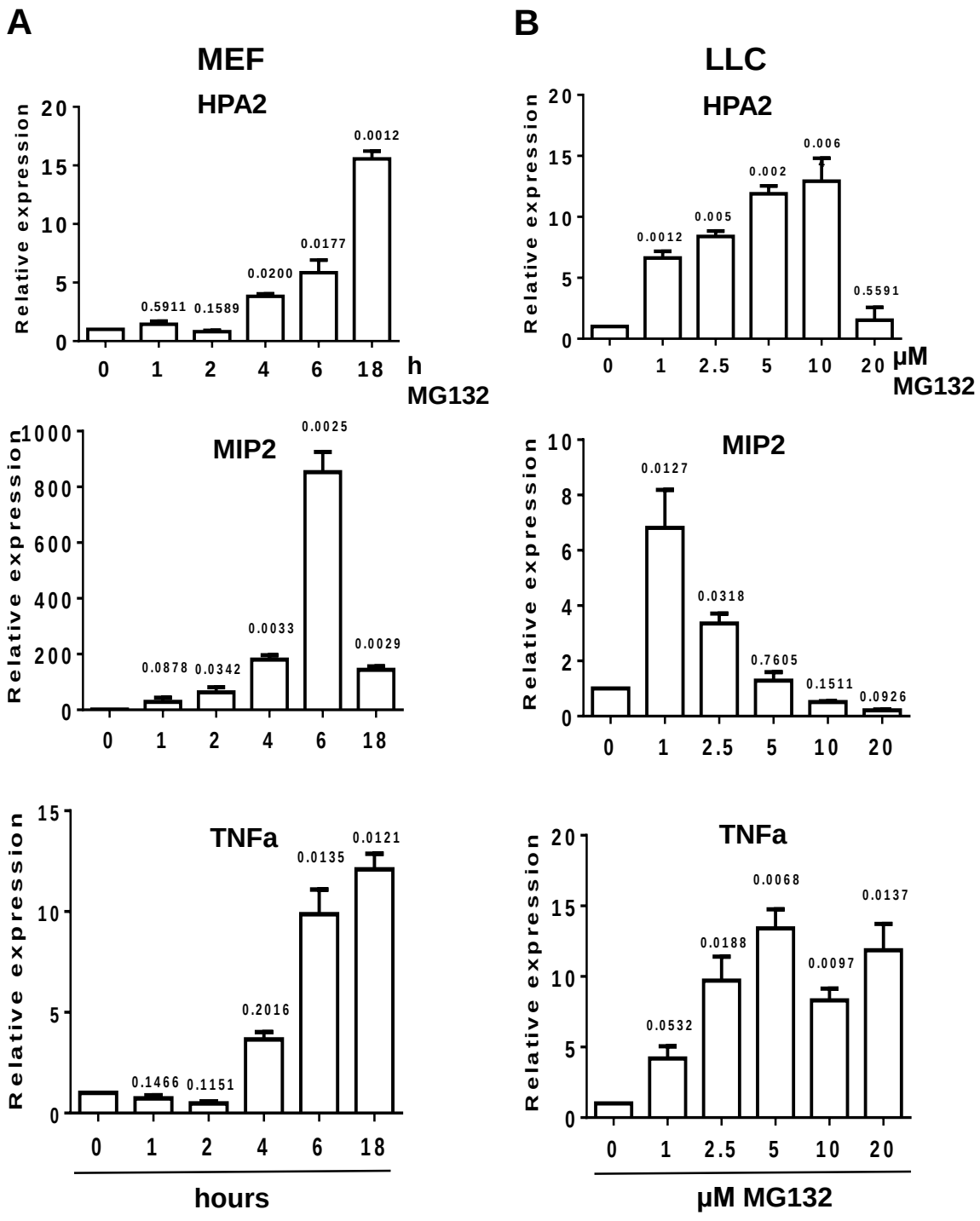
Suppl. Figure 3. **A**. Induction of heat shock protein (HSP) expression by MG132 and Bortezomib. BGC-823 cells were treated with vehicle alone (DMSO, con), MG132 (20 μM), or Bortezomib (0.5 mM) for 16 hours. Total RNA was then extracted and subjected to qPCR applying primers specific for HSP40 (upper panel), HSP105 (second panel), HSP27 (third panel), or HSP70 (lower panel). **B**. MKN-45 cells were similarly treated with MG132 in the absence (MG) or presence of KRIBB 11 (5 μM; MG+KRBB) or KNK-437 (100 μM; MG+KNK) for 16 hours. Total RNA was then extracted and subjected to qPCR applying primers specific for HSP40 (upper panel), HSP105 (second panel), HSPA1A (third panel) and HSPA1B (lower panel). Note that, unlike HSP40 and HSP105, KRIBB 11 and KNK-437 do not attenuate the induction of HSPA1A and HSPA1B by MG132. **: statistically significant decrease of Hpa2 expression in MG+KRBB/MG+KNK vs MG alone.

Suppl. Figure 4. Metformin enhances AMPK phosphorylation, attenuates the proliferation of gastric carcinoma cells, and induces Hpa2 expression. **A**. Inhibitors. MKN-45 cells were

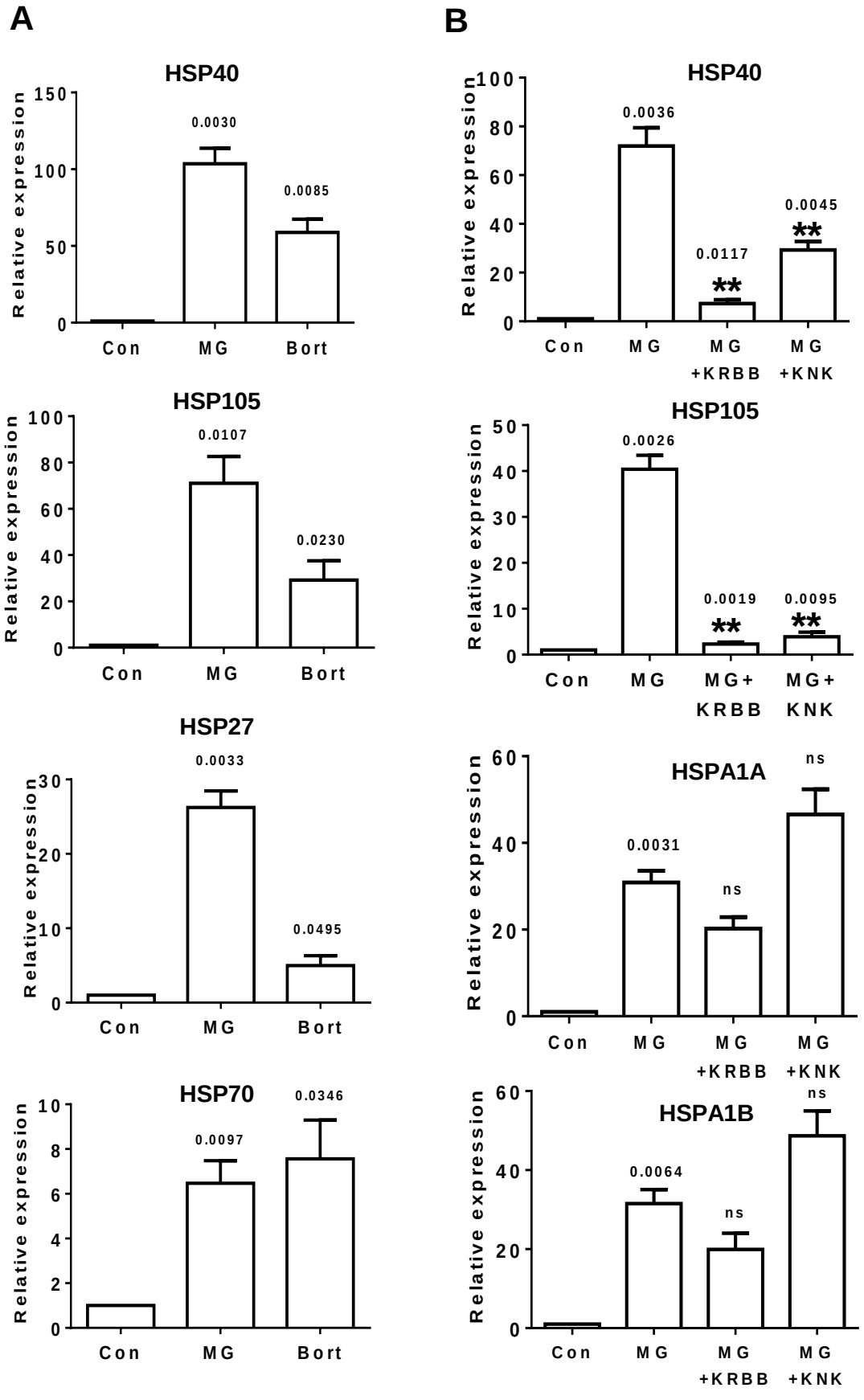
treated with vehicle (DMSO; Con) or MG132 without (Control) or with (+MG) the indicated concentrations of sp600125 (JNK inhibitor), KRIBB 11 (KRBB; HSF1 inhibitor), and dorsomorphin (Dor; AMPK inhibitor). Total RNA was extracted after 24 hours and subjected to qPCR applying primers specific for Hpa2. Note that Hpa2 induction by MG132 is only modestly inhibited by sp600125. **B**. MKN-45 (left) and SGC-7901 (right) cells were treated with metformin (0.5 mM) for the time indicated. Protein extracts were then prepared and subjected to immunoblotting applying anti-phospho-AMPK (upper panels), AMPK (second panels), phospho-ACC (third panel) and ACC (lower panel). MKN-45 cells were treated with the indicated concentration of metformin and relative cell numbers were evaluated as described under 'Materials and methods' (**C**). **D**. MKN-45 cells were treated with phenformin (analog of metformin that reaches higher concentration inside cells; 0.5 mM) for the time indicated. Total RNA was then extracted and subjected to qPCR applying primers specific for Hpa2. **E**. Cellular invasion by control (Vo), Vo+metformin (0.5 mM), and Hpa2 overexpressing SGC-7901 cells were evaluated. Shown are representative images of invading cells taken 24 hours after cell seeding. Quantifications of the number of invading cells is shown graphically in the right panel. **F**. Tumor xenografts produced by control (Vo) and Hpa2 overexpressing FaDu pharyngeal carcinoma cells were subjected to immunostaining applying anti-phospho-AMPK antibody. Shown are representative photomicrographs at low (x25; upper panels) and high (x100; lower panels) magnifications.


A
MKN-45
BGC-823
SGC-7901
MGC-803
p=0.0002
p=0.0001
p=0.0002
p=0.0001
Hpa2
Relative expression
V0
HPA2
B
Ascites fluids (ml)
p=0.04
*
Vo
Hpa2
C
Vo
Hpa2
x10
x100

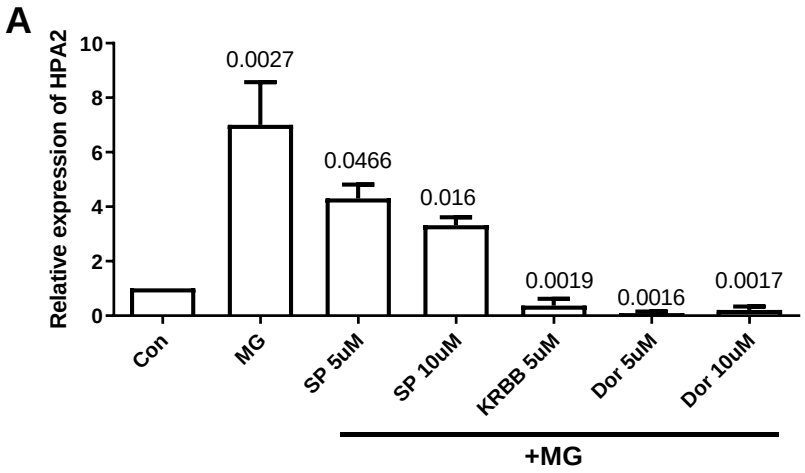
Suppl. Fig. 1

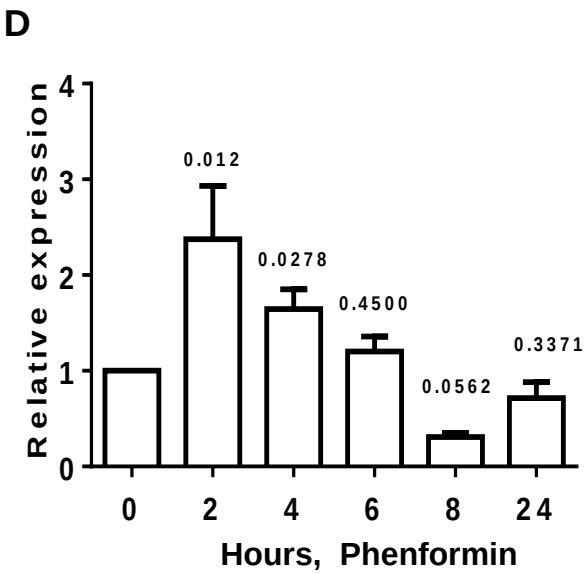

A
MEF
HPA2
Relative expression
20
15
10
5
0
0.5911
0.1589
0.0200
0.0177
0.0012
0
1
2
4
6
18
h
MG132
MIP2
1000
800
600
400
200
0
0.0878
0.0342
0.0033
0.0025
0.0029
TNFa
15
10
5
0
0.1466
0.1151
0.2016
0.0135
0.0121
hours
B
LLC
HPA2
0.0012
0.005
0.002
0.006
0.5591
0
1
2.5
5
10
20
μM
MG132
MIP2
10
8
6
4
2
0
0.0127
0.0318
0.7605
0.1511
0.0926
TNFa
20
15
10
5
0
0.0532
0.0188
0.0068
0.0097
0.0137
μM MG132

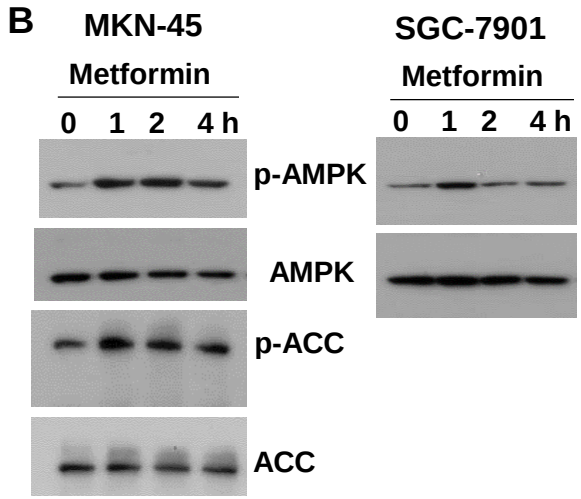
Suppl. Fig. 2

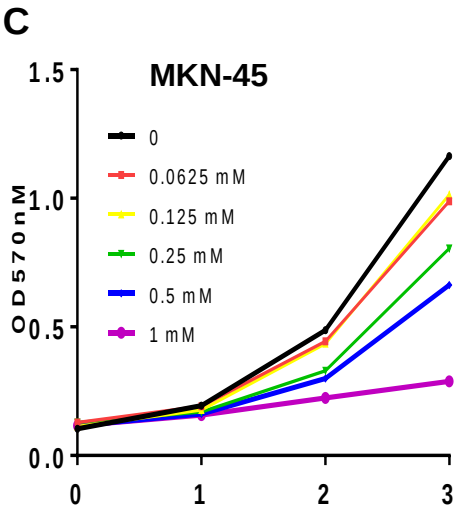

A
B
HSP40
Relative expression
150
100
50
0
0.0030
0.0085
Con
MG
Bort
HSP105
100
80
60
40
20
0
0.0107
0.0230
HSP27
30
20
10
0
0.0033
0.0495
HSP70
10
8
6
4
2
0.0097
0.0346
HSP40
100
80
60
40
20
0.0036
0.0117
0.0045
**
MG +KRBB
MG +KNK
HSP105
50
40
30
10
0.0026
0.0019
0.0095
MG+ KRBB
MG+ KNK
HSPA1A
ns
0.0031
HSPA1B
0.0064

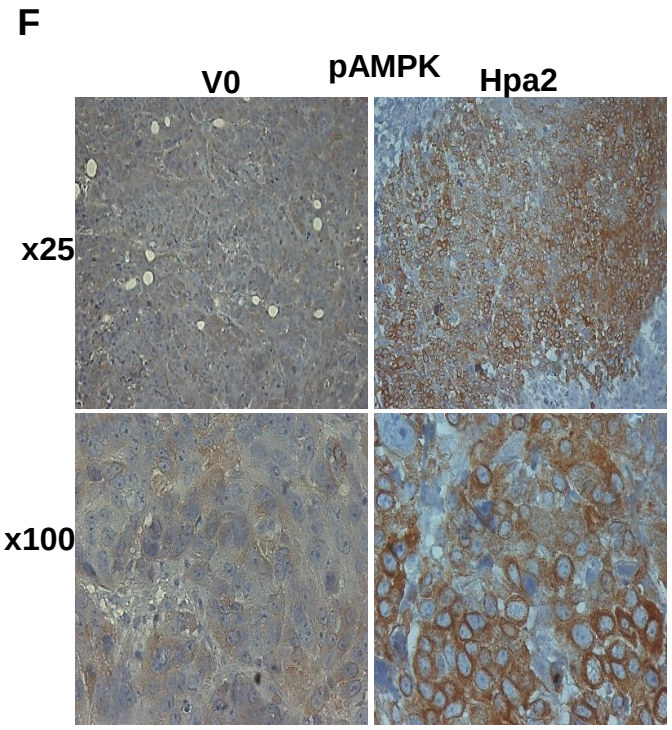
Suppl. Fig. 3

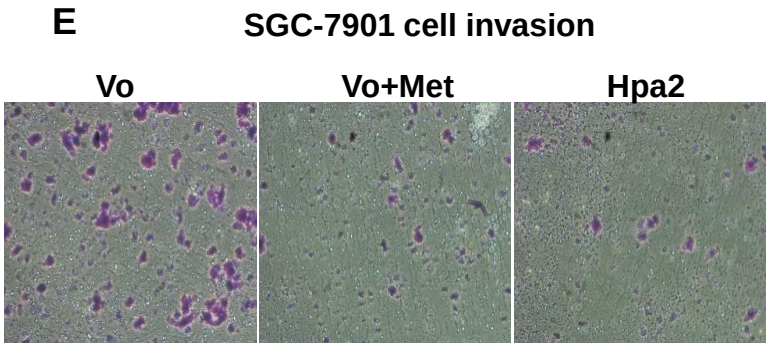

A
Relative expression of HPA2
10
8
6
4
2
0
0.0027
0.0466
0.016
0.0019
0.0016
0.0017
Con
MG
SP 5uM
SP 10uM
KRBB 5uM
Dor 5uM
Dor 10uM
+MG

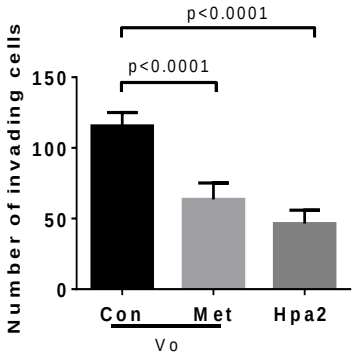

D
Relative expression
4
3
2
1
0
0.012
0.0278
0.4500
0.0562
0.3371
0
2
4
6
8
24
Hours, Phenformin


B
MKN-45
Metformin
0 1 2 4 h
SGC-7901
Metformin
0 1 2 4 h
p-AMPK
AMPK
p-ACC
ACC


C
MKN-45
OD570nM
1.5
1.0
0.5
0.0
0
0.0625 mM
0.125 mM
0.25 mM
0.5 mM
1 mM
0
1
2
3


F
V0
pAMPK
Hpa2
x25
x100


E
SGC-7901 cell invasion
Vo
Vo+Met
Hpa2


Number of invading cells
150
100
50
0
p<0.0001
p<0.0001
Con
Met
Hpa2
Vo

Suppl. Fig. 4

Suppl. Table 1. Sequence of primers sets used in qPCR analyses.

Name	Forward (5'-3')	Reverse
Mouse		
Hpa2	CCTGGAACAGTTCTAGTGCCC	ATGCTCCGATAGTTATTTGGCTC
Actin	ATGCTCCCCGGGCTGTAT	CATAGGAGTCCTTCTGACCCATTC
MIP2	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
TNF alpha	TCAGCCTCTTCTCATTCCTG	TGAAGAGAACCTGGGAGTAG
Human		
Hpa2	CAGGGCATTGATGTCGTGATAC	GCCAGTAGTCTGGTAATGGGTTA
Actin	CGCCCCAGGCACCAGGGC	GCTGGGGTGTTGAAGGT
HSF1	ATAGCCCCAGTAGGACAAACG	TGTGAAGCCCCAACCAAAC
HSP-27	GGACGAGCATGGCTACATCT	GACTGGGATGGTGATCTCGT
HSP-40	AAGAAGCAAGATCCCCCAGT	GCTGGAATGTTGTTGGAGGT
HSP-70	AGCCAAGAAGGCAAAAGTGA	CCACTGCGTTCTTAGCATCA
HSPA1A	CGACCTGAACAAGAGCATCA	AAGATCTGCGTCTGCTTGGT
HSPA1B	CGACCTGAACAAGAGCATCA	AAGATCTGCGTCTGCTTGGT
HSP105 α	ACCACCAGAAAACCCAGACA	TTTGCTTTGTCAGCATCTGG
HSP105 β	TCCGGAAAGATGAACAGGTC	TGCACATCCTCTGGCTACTG