Supporting information to:

Regulation of HL-1 cardiomyocyte apoptosis by EphA2 receptor tyrosine kinase phosphorylation and protection by lithocholic acid

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Supplementary Table

Table S1 Overview: primary antibodies used in this study.

Antibody (order number; manu-facturer)	Antigen, amino acid alignment, description (where available)	Species specificity according to the manufacturer	References reporting antibody use in indicated murine tissue/cells
Caspase 3 (9665; Cell Signaling)	Human: DTIYPRNPAMYSEEARLKSFQNWPD Mouse: DTIYPRNPAMCSEEARLKSFQNWPD Monoclonal antibody produced by immunizing rabbits with a synthetic peptide corresponding to amino-terminal residues adjacent to Asp175 (highlighted in yellow) in human caspase-3. Differences between human and murine sequences are marked in red.	Human, mouse, rat, monkey	Wright *et al.*, 2004 (fibroblasts); Wright *et al.*, 2007 (neurones)
Cleaved caspase 3 (9661; Cell Signaling)	Human: DTIYPRNPAMYSEEARLKSFQNWPD Mouse: DTIYPRNPAMCSEEARLKSFQNWPD Polyclonal antibodies produced by immunizing rabbits with a synthetic peptide corresponding to amino-terminal residues adjacent to Asp175 (highlighted in yellow) in human caspase-3. This antibody does not recognize full length caspase-3 or other cleaved caspases. Differences between human and murine sequences are marked in red.	Human, mouse, rat, monkey	Garnier *et al.*, 2003 (neurones)
EphA2 (sc-924; Santa Cruz)	Rabbit polyclonal antibody against an epitope mapping at the C-terminus of murine EphA2 (no specific information provided).	Mouse, rat, human, mink	Xu *et al.*, 2005 (kidney)
FAK (ab40794; Abcam)	Human: TILEEEKAQQEERMRMESRRQATVSWDSGGS DEAPPKPSRPGYPSPRSSEG (corresponding to amino acid positions 700-750) Mouse: TILEEEKVQQEERMRMESRRQATVSWDSGGS DEAPPKPSRPGYPSPRSSEG (corresponding to amino acid positions 628-678) Rabbit monoclonal antibody produced in rabbits against a synthetic peptide corresponding to non-disclosed residues located within amino acids 700-750 in human FAK. Differences between human and murine sequences are marked in red.	Human, mouse, rat	Ma *et al.*, 2010 (colonic epithelial cells)

GADD153 (ab11419; Abcam)	Mouse monoclonal to GADD153, directed against bacterially expressed mouse CHOP (GADD153) fusion protein.	Mouse, Rat, Human	Nishina *et al*., 2010 (liver)
GAPDH (G8140-11(A); US-Biological)	Human: STHGKFHGTVKAENGKLVINGNPITIFQERDPSKIKWGDAGAEYVVESTG VFTTMEKAGAHLQGGAKRVIISAPSADAPMFVMGVNHEKYDNSLKIISNA SCTTNCLAPLAKVIHDNFGIVEGLMTTVHAITATQKTVDGPSGKLWRDGR GALQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTANVSVVDLTCRLE KPAKYDDIKKVVKQASEGPLKGILGYTEHQVVSSDFNSDT (corresponding to amino acid positions 51-290) Mouse: STHGKFNGTVKAENGKLVINGKPITIFQERDPTNIKWGEAGAEYVVESTG VFTTMEKAGAHLKGGAKRVIISAPSADAPMFVMGVNHEKYDNSLKIVSNA SCTTNCLAPLAKVIHDNFGIVEGLMTTVHAITATQKTVDGPSGKLWRDGR GAAQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTPNVSVVDLTCRLE KPAKYDDIKKVVKQASEGPLKGILGYTEDQVVSCDFNSNS (corresponding to amino acid positions 49-298) Mouse monoclonal antibody. Immunogen: synthetic peptide corresponding to amino acids 51-290 of human GAPDH. Differences between human and murine sequences are marked in red.	Human, mouse, rat	Lugenbiel *et al*., 2012 (HL-1 cells)
Phospho-Akt (Ser473) (9271; Cell Signaling)	Polyclonal antibodies produced by immunizing rabbits with a synthetic phosphopeptide corresponding to residues surrounding Ser473 of mouse Akt.	Mouse, human, rat, hamster, fruit fly, bovine, dog, pig	Paling *et al*., 2004 (embryonic stem cells)
Phospho-EphA2 (Tyr594) (CB4368; Cell Applications)	Human: VYFSKSEQLKPLKTYVDPHTYEDPNQAVLKFTTEIHPSCVT Mouse: VRFSKSEQLKPLKTYVDPHTYEDPNQAVLKFTTEIHPSCVA Polyclonal antibodies produced in rabbit. Antigen: KLH-conjugated peptide containing human EphA2 Tyr594 (highlighted in yellow) and surrounding sequence. Differences between human and murine sequences are marked in red.	Human, mouse, rat	
Phospho-p38 MAPK (Thr180 / Tyr182) (9211; Cell Signaling)	Human: LKPSNLAVNEDCELKILDFGLARHTDDEMTGYVATRWYRAPEIMLNWMHY NQTVDIWSVGCIMAELLTGRTLFPGTDHINQLQQIMRLTGTPPAYLINRM Mouse: LKPSNLAVNEDCELKILDFGLARHTDDEMTGYVATRWYRAPEIMLNWMHY NQTVDIWSVGCIMAELLTGRTLFPGTDHINQLQQIMRLTGTPPAYLINRM Polyclonal antibodies produced by immunizing rabbits with a synthetic phosphopeptide corresponding to residues around Thr180/Tyr182 (highlighted in yellow) of human p38 MAPK.	Human, mouse, rat, monkey, fruit fly, pig	Kawagoe *et al*., 2008 (macrophages)

Phospho-SHP-2 (Tyr542) (3751; Cell Signaling)	Human: IETLQRRIEEEQKSKRKGHEYTNIKYSLADQTSGDQSPLPP Mouse: IETLQRRIEEEQKSKRKGHEYTNIKYSLVDQTSGDQSPLPP Polyclonal antibodies produced by immunizing rabbits with a synthetic phosphopeptide corresponding to residues surrounding Tyr542 (highlighted in yellow) of human SHP-2 protein. Differences between human and murine sequences are marked in red.	Human, mouse, rat	Pan *et al*., 2008 (lacrimal gland)
SHP-2 (3752; Cell Signaling)	Human: MTSRRWFHPNITGVEAENLLLTRGVDGSFLARPSKSNPGDFTLSVRRNGA VTHIKIQNTGDYYDLYGGEKFATLAELVQYYMEHHGQLKEKNGDVIELKY PLNCADPTSERWFHGHLSGKEAEKLLTEKGKHGSFLVRESQSHPGDFVLS VRTGDDKGESNDGKSKVTHVMIRCQELKYDVGGGERFDSLTDLVEHYKKN PMVETLGTVLQLKQPLNTTRINAAEIESRVRELSKLAETTDKVKQGFWEE (corresponding to amino acid positions 1-250) Mouse: MTSRRWFHPNITGVEAENLLLTRGVDGSFLARPSKSNPGDFTLSVRRNGA VTHIKIQNTGDYYDLYGGEKFATLAELVQYYMEHHGQLKEKNGDVIELKY PLNCADPTSERWFHGHLSGKEAEKLLTEKGKHGSFLVRESQSHPGDFVLS VRTGDDKGESNDGKSKVTHVMIRCQELKYDVGGGERFDSLTDLVEHYKKN PMVETLGTVLQLKQPLNTTRINAAEIESRVRELSKLAETTDKVKQGFWEE (corresponding to amino acid positions 1-250) Polyclonal antibodies produced by immunizing rabbits with a synthetic peptide corresponding to non-disclosed residues surrounding the amino-terminal domain of human SHP-2.	Human, mouse, rat	

Akt, protein kinase B; EphA2, erythropoietin-producing human hepatocellular carcinoma receptor tyrosine kinase A2; FAK, focal adhesion kinase; GADD153, growth arrest and DNA damage inducible gene 153; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; SHP-2, Src homology domain-containing protein tyrosine phosphatase 2

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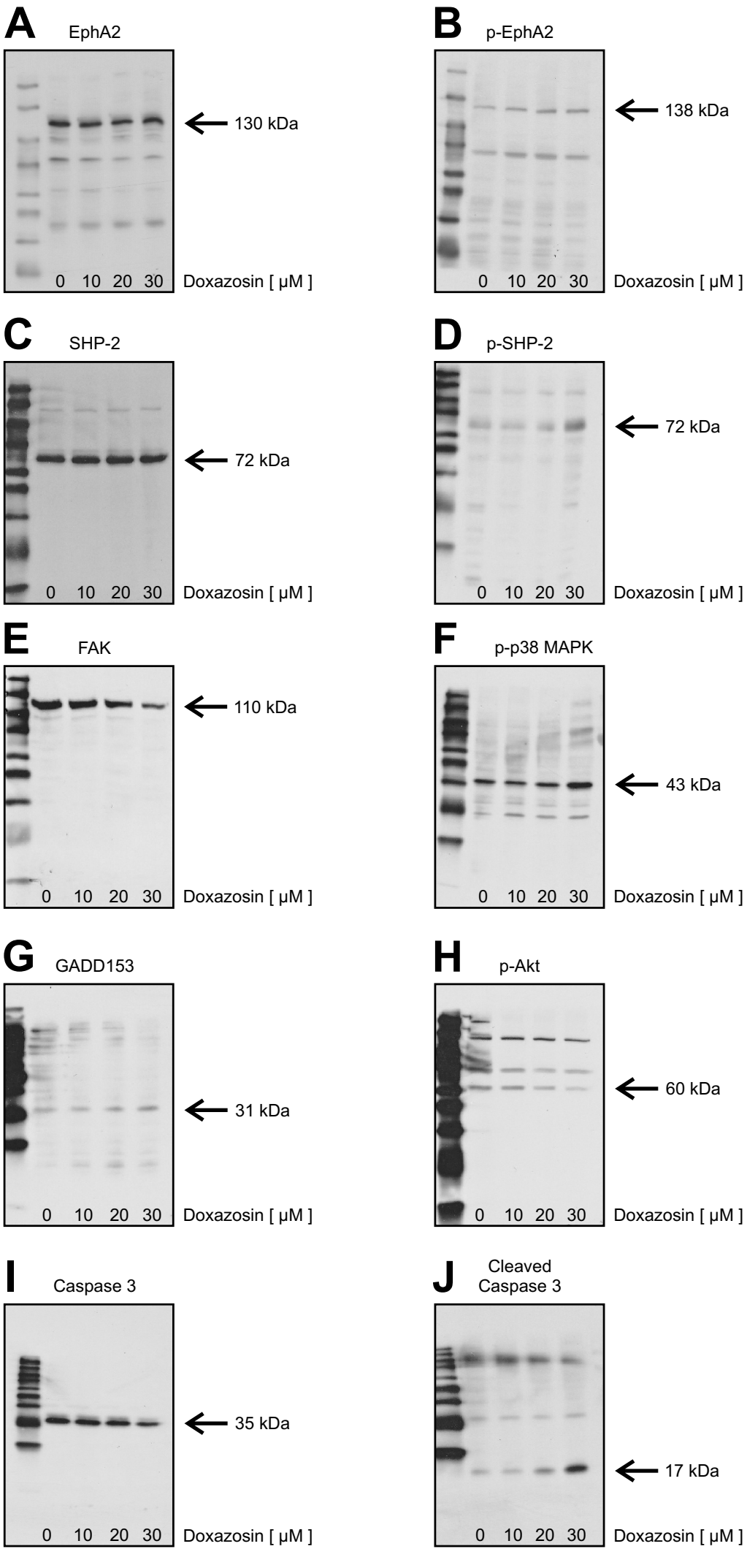
Supplementary Figure legend

Figure S1 Original Western blots corresponding to protein analysis displayed in Figure 2. Apoptosis was associated with increased activation/phosphorylation of erythropoietin-producing human hepatocellular carcinoma receptor tyrosine kinase A2 (EphA2; B), Src homology domain-containing protein tyrosine phosphatase 2 (SHP-2; D), and p38 mitogen-activated protein kinase (MAPK; F), while total EphA2 (A) and SHP-2 (C) levels were not significantly affected. Focal adhesion kinase (FAK) exhibited reduced expression owing to cleavage (E), and growth arrest and DNA damage inducible gene 153 (GADD153) protein levels were elevated (G). The anti-apoptotic protein kinase B (p-Akt) was suppressed (H). (I, J) Doxazosin treatment induced cleavage of caspase 3 (I) associated with decreased expression of pre-processed caspase 3 levels (J).

Figure S1


A
EphA2
130 kDa
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Doxazosin [μM]
B
p-EphA2
138 kDa
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Doxazosin [μM]
C
SHP-2
72 kDa
0 10 20 30
Doxazosin [μM]
D
p-SHP-2
72 kDa
0 10 20 30
Doxazosin [μM]
E
FAK
110 kDa
0 10 20 30
Doxazosin [μM]
F
p-p38 MAPK
43 kDa
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Doxazosin [μM]
G
GADD153
31 kDa
0 10 20 30
Doxazosin [μM]
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p-Akt
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I
Caspase 3
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J
Cleaved
Caspase 3
17 kDa
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Doxazosin [μM]