SUPPLEMENTAL FIGURE LEGENDS

Figure S1: **Homology alignment of DDR2 amino acid sequence.** Shown are the amino acid sequences of human DDR2, mouse DDR2 and the closest homologs in zebrafish and C. Elegans. Degree of homology is indicated by the bar graphs under each amino acid and the position of the novel *DDR2* mutations indicated,

Figure S2: **Squamous lung cancer cell lines harboring *DDR2* mutations are sensitive to tyrosine kinase inhibitor treatment.** (a) Proliferation of A549, NCI-H2286, HCC-366 and NCI-H1703 grown for six days in the presence of various concentrations of imatinib. Proliferation is presented relative to untreated cells at the same time point. Standard errors are presented for triplicate samples. (b) Viability measured by trypan blue exclusion in A549, NCI-H2286, HCC-366 and NCI-H1703 cells grown in the indicated concentrations of dasatinib. Data are presented as viability relative to untreated cells at the same time point and represent an average of fifty independently acquired trypan blue images in each of three replicates with standard errors shown. (c) Proliferation of A549, NCI-H2286, HCC-366 and NCI-H1703 cells grown for six days in the presence of nilotinib. Data are presented as above. (d) Proliferation of A549, NCI-H2286, HCC-366 and NCI-H1703 cells grown in the presence of AP24534. Data are presented as above. (e) Immunoblot showing DDR2 expression from cell lines

used in the experiment shown in Figure 2C. DDR2* denotes the “gatekeeper” transgene.

Figure S3: **Ectopic expression of *DDR2* leads to cellular transformation which is sensitive to AP24534 treatment.** (a) Colony formation in soft agar of NIH-3T3 fibroblasts stably expressing the vector alone or wild-type or various mutant forms of *DDR2*. Colony numbers with standard errors are shown for six independent samples. Expression of FLAG-tagged *DDR2* by immunoblotting of the cells used in the transformation assay is shown in the inset and actin serves as a loading control. 3T3 fibroblasts expressing the activating L858R mutation of *EGFR* (EGFR*) are used as a positive control. (b) Time to IL-3 independence is shown for Ba/F3 cells stably expressing vector alone or wild-type or mutant forms of *DDR2.* Expression level of the transgenes and KD DDR2 (K608E) is shown as well as actin. The KD DDR2 was probed on a separate membrane which is indicated by the separation bar. (c) Proliferation at four days of Ba/F3 cells expressing vector only or one of six *DDR2* mutations is shown in cells grown in the presence of imatinib. For the vector control the cells are grown in the presence of IL-3 to maintain viability and in the case of the *DDR2* mutants all cells are IL-3 independent and cultured in the absence of IL-3. Proliferation is shown relative to untreated cells at the same time point for triplicate samples with standard errors. (d) Experiment as above with AP24534.

Figure S4: **DDR2-transformed Ba/F3 cells maintain Src and STAT5 phosphorylation and treatment of Ba/F3 cells expressing mutant forms of *DDR2* with nilotinib or AZD0530 results in minimal toxicity.** (a) Proliferation at four days of Ba/F3 cells expressing vector only or one of six *DDR2* mutations is shown in cells grown in the presence of nilotinib. For the vector control the cells are grown in the presence of IL-3 to maintain viability and in the case of the *DDR2* mutants all cells are IL-3 independent and cultured in the absence of IL-3. Proliferation is shown relative to untreated cells at the same time point for triplicate samples with standard errors. (b) Immunoblots showing the levels of phospho-Src (Y416), phospho-STAT5 (Y694) and actin in Ba/F3 cells expressing mutant forms of *DDR2* or the vector alone from the cell lines shown in Supplementary Fig. 3b. All *DDR2* mutant lines are grown in the absence of IL-3 and the vector is shown in the presence and absence of IL-3. (c) Experiment as above with Ba/F3 cells grown in the presence of AZD0530. (d) Immunoblots of *DDR2* L63V transformed Ba/F3 cells treated for two days with the depicted concentrations of AZD0530, nilotinib (N), AP24534 (AP) or dasatinib (D). The first lane is an untreated sample. Shown are immunoblots probed with antibodies against phospho-Src, phospho-STAT5, FLAG-DDR2 and actin.

Figure S5: **Combination treatment with nilotinib, AP24534 or dasatinib and AZD0530 leads to increased killing of *DDR2* transformed Ba/F3 cells.** (a) Proliferation at four days of Ba/F3 cells expressing vector only or one of six *DDR2* mutations is shown in cells grown in the presence of a fixed concentration

of nilotinib and the depicted amounts of AZD0530. For the vector control the cells are grown in the presence of IL-3 to maintain viability and in the case of the *DDR2* mutants all cells are IL-3 independent and cultured in the absence of IL-3. Proliferation is shown relative to untreated cells at the same time point for triplicate samples with standard errors. (b) Experiment performed as above with AP24534 and AZD0530. (c) Experiment performed as above with dasatinib and AZD0530.

Figure S6: **Correlation of a *DDR2* kinase domain mutation with a clinical and radiographic response to combination therapy with dasatinib and erlotinib.** Sanger sequencing traces obtained from *DDR2* sequencing of a tissue sample from the patient described in the text. The mutation is outlined by the red box.

Figure S7: **Proposed binding mode of dasatinib to the ATP-binding site of DDR2**. A structural model of the kinase domain of DDR2 (residues 545-854) was generated based on the crystal structure of the Abl kinase domain in the active (DFG-in) conformation (PDB code: 3DK6) in complex with a small molecule inhibitor. Dasatinib (grey mesh) is modeled into the ATP-binding site of DDR2 based on the crystal structure of dasatinib in complex with cSrc (PDB code: 3G5D). The proposed binding mode shows the inhibitor core within hydrogen bonding distance to the backbone of the hinge region (pink) of the kinase domain. The terminal ethanol-piperazine is solvent exposed. The activating mutation Ser768Arg is found at the N-terminal end of a helix (green) below the

activation loop (yellow). The structural model indicates that the side chain of Ser768 (green and red spheres) in wild type DDR2 resides in a packed environment flanked by Glu213 and Phe220. The extra charge and steric bulk of Arg768 in mutant DDR2 is likely to introduce structural changes in this region which could potentially alter the kinase activity and/or regulatory mechanisms of DDR2.