Supplemental Information

Csk-Homologous Kinase (Chk/Matk): a key member of the molecular police force suppressing cancer formation and progression

Gahana Advani, Anderly C. Chueh, Ya Chee Lim, Amardeep Dhillon and Heung-Chin Cheng

Supplemental Table S1

Supplemental Table S2

Supplemental Figure S1

Table S1 Down-regulation of Chk/Matk expression in tumour biopsies and paired normal tissues

Data summarised in the listed references are presented in selected datasets such as Oncomine, NCI Genomic Datasets for Cancer Research.

Cancer types and tissues	Comments	References
Glioblastoma and oligodendroglioma	Down in glioblastoma vs Normal; Down in oligodendroglioma vs normal	Sun, *et al.* (2006) *Cancer Cell* 9:287-300
Glioma	Downregulation of Chk/Matk expression correlates with hypermethylation of its promoter	Laffaire, *et al.* (2011) *Neuro-oncology* 13: 84-98
Gastric cancer	Down in gastric mixed adenocarcinoma vs normal; Down in gastrointestinal stromal tumour vs normal	Cho, *et al.* (2011) *Clin Cancer Res.* 17:1850-1857
Lung cancer	Down in lung carcinoid tumour vs normal; Down in Small cell lung carcinoma vs normal; lung metastasis	Bhattacharjee, *et al.* (2001) *Proc Natl Acad Sci U S A.* 98:13790-13795
Breast cancer	Down in invasive breast carcinoma (progesterone receptor positive)	Chang, *et al.* (2003) *Lancet* 362:362-369.
Colorectal cancer	Down in rectal/colon/cecum adenocarcinomas vs normal	Cancer Genome Atlas Network (2012) *Nature* 2012 487:330-7
	Downregulation in colorectal cancer corregulates with hypermethylation of *Chk/Matk* promoter	Hinoue, *et al.* (2012) *Genome Research* 22:271-282
	Down in Colorectal carcinoma vs normal	Hong, *et al.* (2010) *Clin Exp Metastasis* 27:83-90.
	Down in colon/rectal adenoma vs normal	Sabates-Bellver *et al.* (2007) *Mol Cancer Res* 5:1263-1275.
	Down in colon adenoma vs normal; Down in colon carcinoma vs normal	Skrzypczak, *et al.* (2010) *PLoS One* 5: e13091

Table S2 Predicted effects of the cancer-associated missense mutations on the kinase activity and function of Chk/Matk

Missense mutation (AA)	Primary tissues	Location	Predicted effect on Chk/Matk function
P23S	Skin	N-terminal segment of $p56^{CHK}$ isoform	
S26R	Breast	N-terminal segment of $p56^{CHK}$ isoform	
D73G	Endometrium	SH3 domain	Affect ligand binding to the SH3 domain as this residue is near the RT loop which may contain the high affinity ligand binding sites.
R88L	pancreas	SH3 domain	Affect ligand binding to the SH3 domain as this residue is near one the conserved hydrophobic aromatic residues for binding to PxxP motif
V89I	Large intestine	SH3 domain	Affect ligand binding to the SH3 domain as this residue is near one the conserved hydrophobic aromatic residues for binding to PxxP motif
G95R	Lung	SH3 domain	
A104V	CNS	SH3 domain	
E107K	Skin	SH3 domain	
D114Y	Thyroid	SH3-SH2 connector	
P121L	Lung	SH3-SH2 connector	
F144S	Lung	SH2 domain	The mutation is near the βB strand of the SH2 domain. This strand contains the conserved "FLVRE" motif critical for binding the phosphotyrosine of the phosphotyrosine-containing ligands. The mutation is expected to perturb the ability of CHK SH2 domain to bind its ligands.
S149Y	CNS	SH2 domain	
A150T	Pancreas	SH2 domain	
R151L	Large Intestine	SH2 domain	
D155N	Large Intestine	SH2 domain	
D165N	Large Intestine	SH2 domain	The mutation is near the βD strand of the SH2 domain, which contains the ligand-binding motif. The mutation is expected to perturb the ability of CHK SH2 domain to bind its ligands.
V166I	Pancreas	SH2 domain	
R151H	Oesophagous	SH2 domain	
D199N	Skin	SH2 domain	
A202V	Haematopoietic & lymphoid	SH2 domain	
P210Q	Prostate	SH2 domain	
S218L	kidney	SH2-kinase linker	
G227S	Urinary tract	SH2-kinase linker	

Q240H	Large Intestine	Kinase domain	
E243A	Stomach	Kinase domain	The mutation is within the glycine rich loop, which is essential for binding ATP. This mutation is expected to affect the kinase activity of CHK.
R290H	Stomach	Kinase domain	
F273C	Large Intestine	Kinase domain	The mutation is in αC helix, which contains the conserved Glu-276 critical to catalysis. Glu-276 can electrostatically interact with Lys-262 that binds the β-phosphate of ATP. This mutation is expected to significantly reduce the kinase activity of CHK.
F273V	CNS	Kinase domain	The mutation is in αC helix, which contains the conserved Glu-276 critical to catalysis. Glu-276 can electrostatically interact with Lys-262 that binds the β-phosphate of ATP. This mutation is expected to significantly reduce the kinase activity of CHK.
T277M	CNS	Kinase domain	The mutation is located right next to the conserved Glu-276 in the αC helix. Glu-276 can electrostatically interact with Lys-262 that binds the β-phosphate of ATP. This mutation is expected to significantly reduce the kinase activity of CHK.
H306Q	Lung	Kinase domain	
R319W	Large Intestine	Kinase	
F332C	Endometrium	Kinase domain	
E338Q	Urinary tract	Kinase domain	
A354T	Ovary	Kinase domain	This mutation is mapped to the catalytic loop (HRDL**A**ARN) critical to catalysis. This mutation is expected to inactivate or abolish the catalytic activity.
R356H	Stomach	Kinase domain	This mutation is mapped to the catalytic loop (HRDLAA**R**N) critical to catalysis. This mutation is expected to inactivate or abolish the catalytic activity.
V368I	Large Intestine	Kinase domain	This mutation is near the catalytic loop (HRDLAARN) critical to catalysis. This mutation is expected to inactivate or abolish the catalytic activity.
D370N	Endometrium	Kinase domain	Asp-370 in the activation loop is critical to binding Mg^{++}- ATP. Its mutation is expected to significantly reduce the kinase activity of CHK.
S384N	Large intestine	Kinase domain	This mutation, mapped to the activation loop, is expected to significantly reduce the kinase activity of CHK.
R385Q	Large Intestine	Kinase domain	This mutation, mapped to the activation loop, is expected to significantly reduce the kinase activity of CHK.
R385W	Stomach	Kinase domain	This mutation, mapped to the activation loop, is expected to significantly reduce the kinase activity of CHK.
K389E	Endometrium	Kinase domain	This mutation, mapped to the activation loop, is expected to significantly reduce the kinase activity

			of CHK.
W390C	Large intestine	Kinase domain	This mutation, mapped to the activation loop, is expected to significantly reduce the kinase activity of CHK.
A392V	Large Intestine	Kinase domain	This mutation, mapped to the activation loop, is expected to significantly reduce the kinase activity of CHK.
P393L	Large Intestine	Kinase domain	As Pro-393 is the activation loop residues involved in binding the protein substrate (referred to as the P+1 loop). Its mutation is expected to significantly reduce the kinase activity of CHK.
E416A	Lung	Kinase domain	This mutation is mapped to the F-helix. As movement of the F-helix governs
F418L	Lung	Kinase domain	F-helix
P424S	Skin	Kinase domain	This mutation is mapped to one of the Src-recognition loop. The mutation may perturb the binding of Chk/Matk to SFKs.
S434L	Large Intestine	Kinase domain	
R442C	Skin	Kinase domain	
E447K	Endometrium	Kinase domain	
V453L	Lung	Kinase domain	
R467H	Endometrium	Kinase domain	
P469S	Breast	Kinase domain	
A496T	Large Intestine	Kinase domain	
R503Q	Large Intestine	C-terminal Tail	

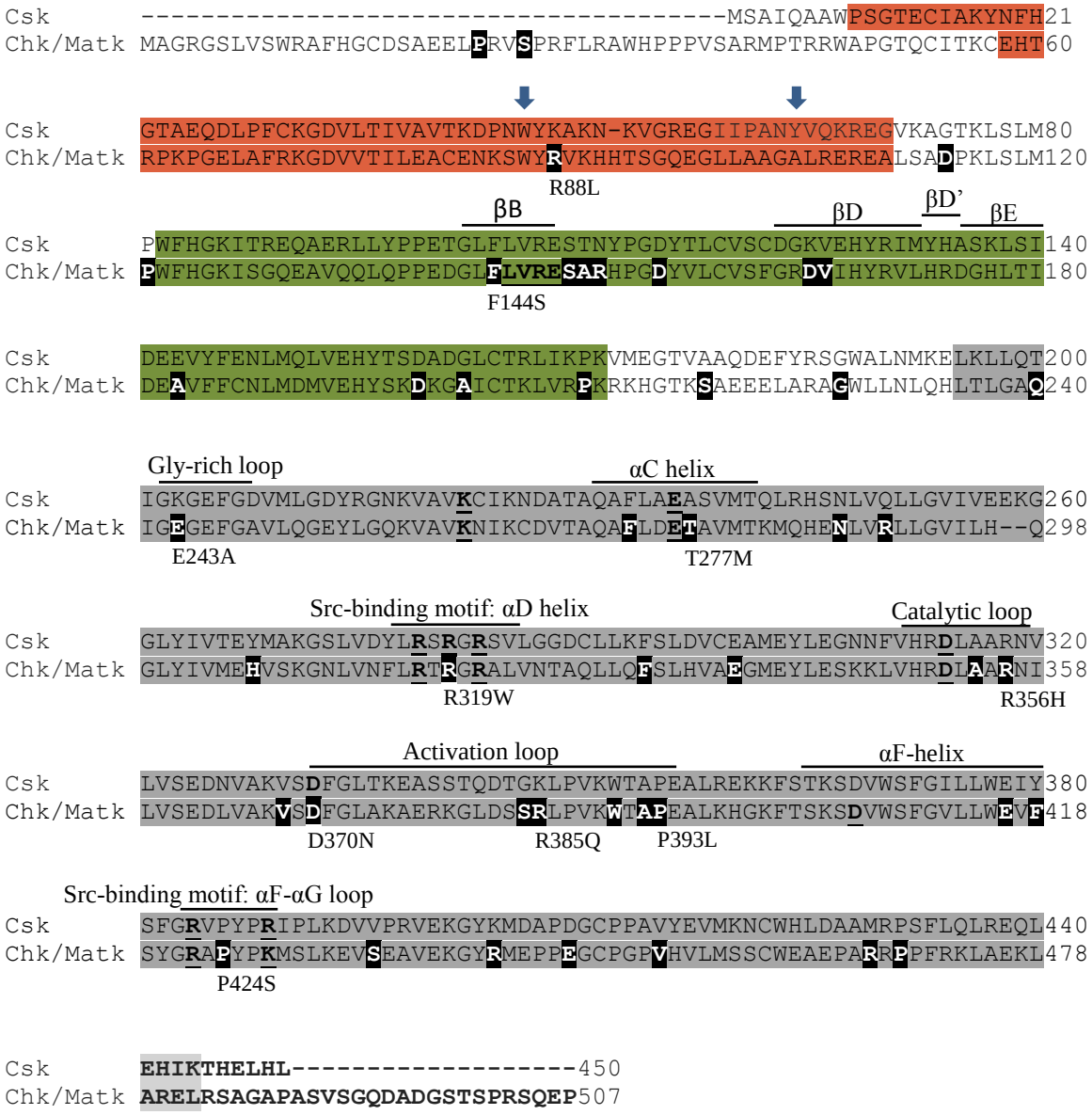

Figure S1 Alignment of the sequences of human Csk and Chk/Matk (p56$^{Chk/Matk}$)

The sequences of the SH3, SH2 and kinase domains are colour-coded in red, green and grey, respectively. Arrows indicate aromatic hydrophobic residues in SH3 domain for binding to PxxP motif of ligands. The amino acid residues shown in white font against the black background are residues mutated in cancer. Among them, those located at motifs that are potentially critical to catalysis and SFK recognition are shown in Fig. 2C. These mutated residues are presented as E243A, T277M, R319W, etc.