Supplementary Figure Legends

Supplementary Figure 1 - Cell-Specific Labeling With Amino Acid Precursors. a) *meso*-2,6-Diaminopimelate (DAP) and *D*-lysine are *L*-lysine precursors that can not be metabolized by eukaryotic cells. Diaminopimelate Decarboxylase (DDC) (PDB: 2QGH) and Lysine Racemase (Lyr) (PDB: 4DZA) are non-mammalian amino acid processing enzymes that can specifically convert their respective amino acid precursors into functional *L*-amino acids. **b)** Conceptually, eukaryotic cells transfected with DDC and Lyr exclusively proliferate on DAP and *D*-lysine respectively. When grown on "Light" DAP and "Heavy" (+8 Da) *D*-lysine, +DDC and +Lyr cells maintain proteomic labeling fidelity in a proliferating co-culture. However, the fidelity of this labeling technique is directly dependent on the performance of DDC and Lyr amino acid processing enzymes. **c)** Optimal DDC and Lyr enzymes should: 1) Confer excellent precursor-specific proliferation, 2) retain the enzymes intracellularly, 3) be applicable to multiple cell types, 4) avoid transcriptional cell stress and 5) facilitate continuous co-culture experiments.

Supplementary Figure 2 - Structural Variation Between DDC Enzymes. a) DDC from *Helicobacter pylori* (PDB: 2QGH) (Green), *Methanocaldococcus jannaschii* (PDB: 1TUF) (Blue) and *Mycobacterium tuberculosis* (PDB: 1HKW) (Red). We hypothesized that screening structurally distinct DDC orthologs would help identify an improved DDC enzyme for use in cell-specific labeling with amino acid precursors. For example, as DDC is catalytically active as a homodimer, we hypothesized that different quaternary structures could affect ectopic DDC activity in mammalian cells. As a result, we screened DDC enzymes that form distinct quaternary crystals: DDC from *H. pylori* crystallizes as a monomer, whereas DDC from *M. jannaschii* and *M. tuberculosis* form homodimers. In contrast, *M. tuberculosis* DDC forms a covalent homodimer through a disulphide bridge between Cys93 and Cys375 (Yellow). **b)** We also hypothesized that variations in active site access could alter ectopic DDC activity in mammalian cells. Each of the screened DDC enzymes has a distinct "Active-Site Loop" responsible for controlling access to the DDC catalytic site (indicated by lysine (Green) and PLP (Magenta)).

Supplementary Figure 3 - Enzyme Leader Sequences. SignalP 4.1 analysis of **a)** DDC and **b)** Lyr amino-terminal sequences. Predictive C-Score (raw cleave site score) (red), S-Score (signal peptide score) (green) and Y-Score (combined cleavage site score) (blue) are shown. High S-scores suggest a signal sequence is present and high C/Y-Scores predict potential cleavage sites. Wild-type Lyr ($\mathrm{Lyr}^{\mathrm{WT}}$) contains a predicted signal peptide (amino acids 1-30) and several cleavage motifs. “Modified Lyr” also contains potential signal elements although this is less pronounced than $\mathrm{Lyr}^{\mathrm{WT}}$. In contrast, $\mathrm{Lyr}^{\mathrm{M37}}$ shows no evidence of a signal sequence motif. In addition, no DDC enzymes contain predicted signal peptide motifs.

Supplementary Figure 4 - $\mathbf{GFP^{+}}$ and $\mathbf{RFP^{+}}$ Co-Culture FACS. a) 10 day MDA-MB-231 / C3H10T1/2 co-cultures were sorted by their GFP and RFP expression. DAPI was used to distinguish between live and dead cells. **b)** Post-FACS cell populations were re-analyzed for GFP^{+} and RFP^{+} purity.

Supplementary Figure 5 - Selected Reaction Monitoring (SRM) of Lyr and DDC. a) Unscheduled SRM of proteotypic Lyr peptides from C3H10T1/2 cells transfected with either empty vector, $\mathrm{Lyr}^{\mathrm{M37\text{-}KDEL}}$ or $\mathrm{DDC}^{M.tub\text{-}\mathrm{KDEL}}$. Proteotypic Lyr product ion transitions are only detected in cells transfected with $\mathrm{Lyr}^{\mathrm{M37\text{-}KDEL}}$. **b)** Unscheduled SRM of proteotypic $\mathrm{DDC}^{M.tub}$ peptides from C3H10T1/2 cells transfected with either empty vector, $\mathrm{Lyr}^{\mathrm{M37\text{-}KDEL}}$ or $\mathrm{DDC}^{M.tub\text{-}\mathrm{KDEL}}$. Proteotypic $\mathrm{DDC}^{M.tub}$ product ion transitions are only detected in cells transfected with $\mathrm{DDC}^{M.tub\text{-}\mathrm{KDEL}}$.

Supplementary Data - Figure 3b. Proteome Discoverer 1.4 grouped peptide view of data from Figure 3b.

Supplementary Data - Figure 4c. Proteome Discoverer 1.4 grouped peptide view of data from Figure 4c.

Annotated Enzyme Sequences

```
A.thaliana          MAAATQFLSQPSSLNPHQLKNQTSQRSRSIPVLSLKSTLKPLKRLSVKAAVVSQNSSKTV 60
H.pylori            -----------------------------------------------------------M 1
M.jannaschii        -----------------------------MKIMFLG-----------NDTVEIKDGRFFI 20
M.tuberculosis      ----------------------------------MNELLHLAPNVWPRNTTRDEVGVVCI 26
M.leprae            ---------MNVHTAGPRHAEKTRHTATPQRVQPSDDLLRLASNVWPRNITRDETGVACI 51
M.avium             ---------MNVHPAGPRHAEETRHPESPPRPQSPEELLLLAPNVWPRNATRNEAGVATI 51
                                                                               :

A.thaliana          TKFDHCFKKSSDGFLYCEGTKVEDIMESVERRPFYLYSKPQITRNLEAYKEALE------ 114
H.pylori            FNYEELFQT--------------------HKTPFYLYDFDKIKQAFLNYKEAFK------ 35
M.jannaschii        DGYDAIELAEK------------------FGTPLYVMSEEQIKINYNRYIEAFKRWEEET 62
M.tuberculosis      AGIPLTQLAQ------------------EYGTPLFVIDEDDFRSRCRETAAAFG------ 62
M.leprae            AGNKLTDLAG------------------EYGTPLFVIDEDDFRFRCREIAAAFG------ 87
M.avium             AGVAVTELAR------------------EYGTPLFVVDEDDFRSRCREIASAFG------ 87
                                                    *::: .  .:         *:

A.thaliana          GVSSVIGYAIKANNNLKILEHLRSLGCGAVLVSGNELRLALRAGFDPTKCIFNGNGKSLE 174
H.pylori            GRKSLICYALKANSNLSILSLLAHLESGADCVSIGEIQRALKAGIKPYRIVFSGVGKSAF 95
M.jannaschii        GKEFIVAYAYKANANLAITRLLAKLGCGADVVSGGELYIAKLSNVPSKKIVFNGNCKTKE 122
M.tuberculosis      -SGANVHYAAKAFLCSEVARWISEEGLCLDVCTGGELAVALHASFPPERITLHGNNKSVS 121
M.leprae            -GGENVHYAAKAFLCTEIARWIDEEGLSLDVCSGGELAVALHASFPPERISLHGNNKSVA 146
M.avium             -GGNNVHYAAKAFLCSEVARWIDEEGLSLDVCTGGELAVALHADFPPERITFHGNNKSVA 146
                         : ** **     :   :          : .*:  *  :.. . :  : *  *:

A.thaliana          DLVLAAQEG-VFVNVDSEFDLNNIVEASRISGKQVNVLLRINPDVDPQVHPYVATGNKNS 233
H.pylori            EIEQALKLNILFLNVESFMELKTIETIAQSLGIKARISIRINPNIDAKTHPYISTGLKEN 155
M.jannaschii        EIIMGIEANIRAFNVDSISELILINETAKELGETANVAFRINPNVNPKTHPKISTGLKKN 182
M.tuberculosis      ELTAAVKAGVGHIVVDSMTEIERLDAIAGEAGIVQDVLVRLTVGVEAHTHEFISTAHEDQ 181
M.leprae            ELKDAVKAGVGYIVLDSTTEIERLDAIAGEAGIVQDVLVRLTVGVEAHTHEFIATAHEDQ 206
M.avium             ELTAAVKAGVGHVVLDSMTEIERLDAIAADAGIVQDVFVRLTVGVEAHTHEFISTAHEDQ 206
                    ::  . : .   . ::*  ::  :   :   *    : .*:. .::.:.*  ::*. :..

A.thaliana          KFGIR-NEKLQWFLDQVKAHPKELKLVGAHCHLGSTITKVDIFRDAAVLMIEYIDEIR-- 290
H.pylori            KFGVGEKEALEMFLWAKKS--AFLEPVSVHFHIGSQLLDLEPIIEASQKVAKIAKSLI-- 211
M.jannaschii        KFGLDVESGIAMKAIKMALEMEYVNVVGVHCHIGSQLTDISPFIEETRKVMDFVVELK-- 240
M.tuberculosis      KFGLSVASGAAMAAVRRVFATDHLRLVGLHSHIGSQIFDVDGFELAAHRVIGLLRDVVGE 241
M.leprae            KFGLSVASGAAMAAVRRVFATDNLRLVGLHSHIGSQIFDVAGFELAAHRVIGLLCDIVGE 266
M.avium             KFGLSVASGSALAAVGRVFDTEHLRLVGLHSHIGSQIFDVAGFELAARRVIGLLHDAVEQ 266
                    ***:   .               :. *. * *:** : .:  :   :  :     .

A.thaliana          ---RQGFEVSYLNIGGGLGIDYYHAGAVLPTPMDLINTVRELVLSRD-------LNLIIE 340
H.pylori            ---ALGIDLRFFDVGGGIGVSYENEETIK--LYDYAQGILNALQGLD-------LTIICE 259
M.jannaschii        ---EEGIEIEDVNLGGGLGIPYYKDKQIP-TQKDLADAIINTMLKYKDKV--EMPNLILE 294
M.tuberculosis      FGPEKTAQIATVDLGGGLGISYLPSDDPP-PIAELAAKLGTIVSDESTAVGLPTPKLVVE 300
M.leprae            FDPEKTAQLSIVDLGGGLGISYLPDDDPP-PIFELAAKLGAIVSNESAAVGLPVPKLMVE 325
M.avium             FGVEKTAQIATVDLGGGLGISYLAADDPP-PMGELAGKLSAIVQHESAAVGLPTPRLVVE 325
                           ::  .::***:*: *           :    :   :   .         :: *

A.thaliana          PGRSLIANTCCFVNHVTGVKTNGT-----KNFIVIDGSMAELIRPSLYDAYQHIELVSPP 395
H.pylori            PGRSIVAESGELITQVLYEKKAQN-----KRFVIVDAGMNDFLRPSLYHAKHAIRVITPS 314
M.jannaschii        PGRSLVATAGYLLGKVHHIKETPV-----TKWVMIDAGMNDMMRPAMYEAYHHIINCKVK 349
M.tuberculosis      PGRAIAGPGTITLYEVGTVKDVDVSATAHRRYVSVDGGMSDNIRTALYGAQYDVRLVSRV 360
M.leprae            PGRAIAGPGTITLYEVGTIKDVDVSATAHRRYVSIDGGMSDNIRTALYDAQYDVRLVSRT 385
M.avium             PGRAIAGPGTITLYEVGTVKDVDVSATAHRRYVSVDGGMSDNIRTALYDAQYDARLVSRV 385
                    ***:: .     : .*   *          .:: :*..* : :*.::* *       .

A.thaliana          PAEAEVTKFDVVGPVCESADFLGKDRELP-TPPQGAGLVVHDAGAYCMSMASTYNLKMRP 454
H.pylori            -KGREISPCDVVGPVCESSDTFLKDAHLP-ELEPGDKIAIEKVGAYGSSMASQYNSRPKL 372
M.jannaschii        ---NEKEVVSIAGGLCESSDVFGRDRELD-KVEVGDVLAIFDVGAYGISMANNYNARGRP 405
M.tuberculosis      -SDAPPVPARLVGKHCESGDIIVRDTWVPDDIRPGDLVAVAATGAYCYSLSSRYNMVGRP 419
M.leprae            -SDAPAAPASIVGKHCESGDIVVRDTWVPDDLKPGDLVGVAATGAYCYSLSSRYNMLGRP 444
M.avium             -SDAPAELARIVGKHCESGDIVVRDTWVPGDLRPGDLIGVAATGAYCYSLSSRYNMVCRP 444
                              :.*  ***.* . :*  :      *  : :  .***  *::. **   :

A.thaliana          PEYWVEEDGSITKIRHAETFDDHLRF----FEGLGSGSGYPYDVPDYA 498
H.pylori            LELALEDH-KIRVIRKREALEDLWRLEEEGLKGVGSGSGYPYDVPDYA 419
M.jannaschii        -RMVLTSKKGVFLIRERETYADLIAKDIVPPHLLGSGSGYPYDVPDYA 452
M.tuberculosis      AVVAVHAGNARLVLR-RETVDDLLSLEVR-----GSGSGYPYDVPDYA 461
M.leprae            AVVAVCAGQARLILR-RETVDDLLSLEVR-----GSGSGYPYDVPDYA 486
M.avium             AVVAVRDGRARLVLR-RETVDDLLSLEVR-----GSGSGYPYDVPDYA 486
                        :        :*  *:  *            **************
```

Annotated DDC Sequences. Active site residues are highlighted in Blue, disulphide-linked cysteines in Yellow. Carboxyl-terminal HA-tag in bold. Proteotypic DDC$^{M.tub}$ SRM peptides are underlined. Alignment performed with ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

```
                >Leader                              >Catalytic Core
Lyr(WT)         MSLGIRYLALLPLFVITACQQPVNYNPPATQVAQVQPAIVNNSWIEISRSALDFNVKKVQ 60
Lyr(M37)        ------------------------------------MAIVNNSWIEISRSALDFNVKKVQ 24
Lyr(M37-KDEL)   ------------------------------------MAIVNNSWIEISRSALDFNVKKVQ 24
                                                     ***********************

Lyr(WT)         SLLGKQSSLCAVLKGDAYGHDLSLVAPIMIENNVKCIGVTNNQELKEVRDLGFKGRLMRV 120
Lyr(M37)        SLLGKQSSLCAVLKGDAYGHDLSLVAPIMIENNVKCIGVTNNQELKEVRDLGFKGRLMRV 84
Lyr(M37-KDEL)   SLLGKQSSLCAVLKGDAYGHDLSLVAPIMIENNVKCIGVTNNQELKEVRDLGFKGRLMRV 84
                ************************************************************

Lyr(WT)         RNATEQEMAQATNYNVEELIGDLDMAKRLDAIAKQQNKVIPIHLALNSGGMSRNGLEVDN 180
Lyr(M37)        RNATEQEMAQATNYNVEELIGDLDMAKRLDAIAKQQNKVIPIHLALNSGGMSRNGLEVDN 144
Lyr(M37-KDEL)   RNATEQEMAQATNYNVEELIGDLDMAKRLDAIAKQQNKVIPIHLALNSGGMSRNGLEVDN 144
                ************************************************************

Lyr(WT)         KSGLEKAKQISQLANLKVVGIMSHYPEEDANKVREDLARFKQQSQQVLEVMGLERNNVTL 240
Lyr(M37)        KSGLEKAKQISQLANLKVVGIMSHYPEEDANKVREDLARFKQQSQQVLEVMGLERNNVTL 204
Lyr(M37-KDEL)   KSGLEKAKQISQLANLKVVGIMSHYPEEDANKVREDLARFKQQSQQVLEVMGLERNNVTL 204
                ************************************************************

Lyr(WT)         HMANTFATITVPESWLDMVRVGGIFYGDTIASTDYKRVMTFKSNIASINYYPKGNTVGYD 300
Lyr(M37)        HMANTFATITVPESWLDMVRVGGIFYGDTIASTDYKRVMTFKSNIASINYYPKGNTVGYD 264
Lyr(M37-KDEL)   HMANTFATITVPESWLDMVRVGGIFYGDTIASTDYKRVMTFKSNIASINYYPKGNTVGYD 264
                ************************************************************

Lyr(WT)         RTYTLKRDSVLANIPVGYADGYRRVFSNAGHALIAGQRVPVLGKTSMNTVIVDITSLNNI 360
Lyr(M37)        RTYTLKRDSVLANIPVGYADGYRRVFSNAGHALIAGQRVPVLGKTSMNTVIVDITSLNNI 324
Lyr(M37-KDEL)   RTYTLKRDSVLANIPVGYADGYRRVFSNAGHALIAGQRVPVLGKTSMNTVIVDITSLNNI 324
                ************************************************************
                                                                >HA-Tag
Lyr(WT)         KPGDEVVFFGKQGNSEITAEEIEDISGALFTEMSILWGATNQRVLVDGYPYDVPDYA--- 417
Lyr(M37)        KPGDEVVFFGKQGNSEITAEEIEDISGALFTEMSILWGATNQRVLVDGYPYDVPDYA--- 381
Lyr(M37-KDEL)   KPGDEVVFFGKQGNSEITAEEIEDISGALFTEMSILWGATNQRVLVDGYPYDVPDYAGSG 384
                *********************************************************

Lyr(WT)         ----
Lyr(M37)        ----
Lyr(M37-KDEL)   KDEL 388
```

Annotated Lyr Sequences. Leader sequence shown in green, catalytic core in blue, HA-tag in bold and KDEL in red. Proteotypic peptides used for Lyr$^{\text{M37-KDEL}}$ SRM are underlined. Catalytic site residues highlighted in yellow.

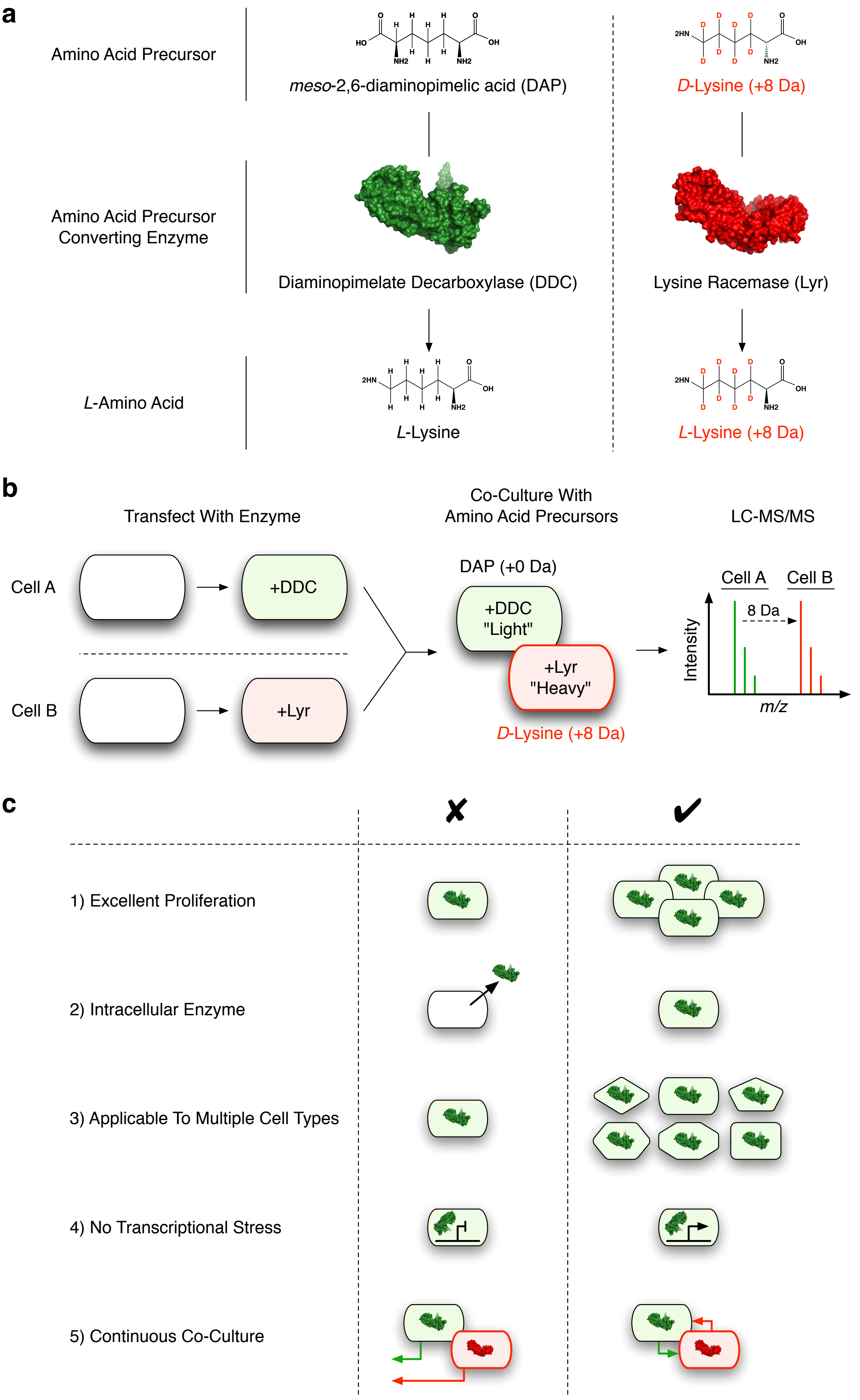
Protein	Sequence	Product	Light		Heavy (SILAC)		Heavy (CTAP)	
			Precursor Mass	Product Mass	Precursor Mass	Product Mass	Precursor Mass	Product Mass
Lyr	**SALDFNVK**	y6	447.240	735.404	451.247	743.418	451.265	743.454
		y5		622.320		630.334		630.370
		y4		507.293		515.307		515.343
		y3		360.224		368.238		368.274
	SNIASINYYPK	y8	635.327	955.488	639.334	963.503	639.352	963.539
		y7		884.451		892.465		892.501
		y6		797.419		805.433		805.469
		y3		407.229		415.243		415.279
	VPVLGK	y5	306.708	513.340	310.715	521.354	310.733	521.390
		y4		416.287		424.301		424.337
		y3		317.218		325.233		325.269
DDC	**SVSELTAAVK**	y8	502.785	818.462	506.792	826.476	506.810	826.512
		y7		731.430		739.444		739.480
		y6		602.387		610.401		610.437
		y5		489.303		497.317		497.353
	DVVGEFGPEK	y8	538.767	862.431	542.774	870.445	542.792	870.481
		y7		763.362		771.376		771.412
		y5		577.298		585.312		585.348
		y4		430.230		438.244		438.280
	LGTIVSDESTAVGLPTPK	y13	892.986	1301.658	896.993	1309.673	897.011	1309.709
		y7		711.440		719.454		719.490
		y6		612.372		620.386		620.422
		y4		442.266		450.280		450.316

Supplementary Table 1 - Proteotypic Lyr and DDC Peptides For Selected Reaction Monitoring (SRM). Product ion values for isotopic variants of proteotypic Lyr and DDC peptides. Product ions used for relative quantification are highlighted in red. All precursors ions are doubly charged and all product ions are singly charged.

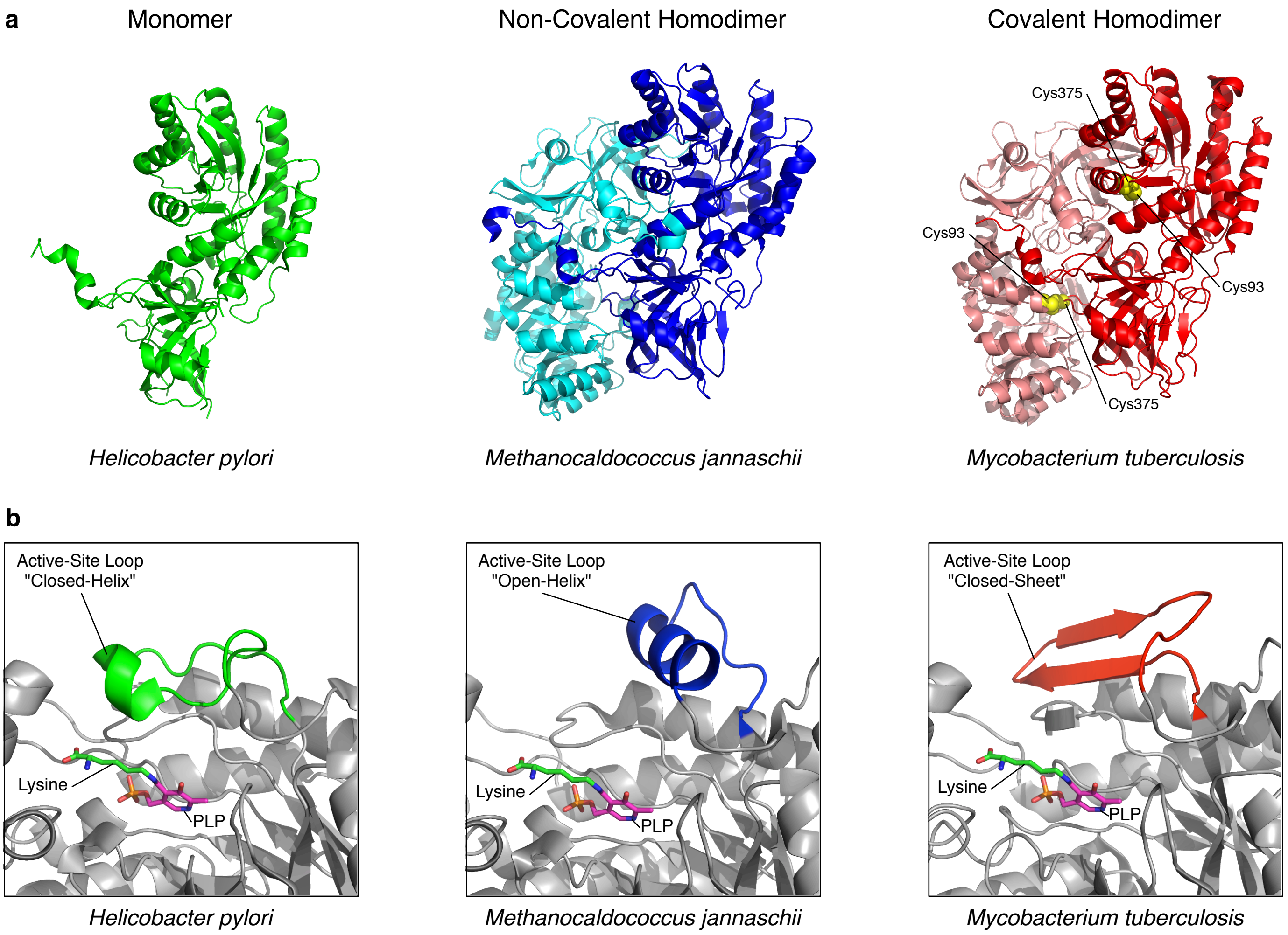
			Interrupted (H) vs. Interrupted (M) (Control)				**Continuous (H) vs. Interrupted (M)**			
Peptide	**Protein (Accession)**	**p-Site**	**H / M Rep. #1**	**H / M Rep. #2**	**H / M Rep. #3**	**H / M Mean**	**H / M Rep. #1**	**H / M Rep. #2**	**H / M Rep. #3**	**H / M Mean**
TA**S**EGSEAETPEAPK	LARP7 (Q05CL8)	Thr 251 Ser 253	0.962	0.999	0.985	0.98	9.467	-	-	**9.47**
IA**S**DEEIQGTK	PALLD (Q9ET54)	Ser 901	0.618	-	0.815	0.72	7.455	-	6.139	**6.80**
RL**SS**LRASTSK	RS6 (P62754)	Ser 235 Ser 236	1.918	0.640	2.097	1.55	7.26	5.37	2.59	**5.07**
SQ**S**KSPTGTPAR	TRA2A (Q6PFR5)	Ser 16 Ser 18	1.002	-	-	1.00	-	-	5.214	**5.21**
RL**S**YNTASNK	RL34 (Q9D1R9)	Ser 12	1.024	-	0.891	0.96	6.685	-	2.970	**4.83**
KR**S**I**S**ESSR	PININ (O35691)	Ser 698 Ser 700	0.722	-	-	0.72	-	-	4.758	**4.76**
KR**S**EGLSLER	HDGR2 (Q3UMU9)	Ser 450	-	-	-	-	4.417	-	4.573	**4.50**
SG**S**SSPDSEITELK	PYRG1 (P70698)	Ser 573	-	-	-	-	-	-	4.408	**4.41**
SRL**T**PT**T**PESSSTGTEDK	SQSTM (Q64337)	Thr 269 Thr 272	1.284	-	-	1.28	4.061	4.726	3.439	**4.08**
RS**S**DTCG**S**PALPSK	AB1IP (Q8R5A3)	Ser 532 Ser 537	-	-	-	-	-	-	3.308	**3.31**
EPQ**S**PS**S**QSTPCKPTNDR	SPAT5 (Q3UMC0)	Ser 244 Ser 247	-	-	-	-	-	-	3.178	**3.18**
SLENETLNK	EIF4B (Q8BGD9)	Ser 445	-	1.085	1.250	1.17	2.364	3.211	2.137	**2.57**
SSPNVANQPP**S**PGGK	AFAD (Q9QZQ1)	Ser 1182	-	-	-	-	-	-	2.290	**2.29**
GQLHGS**S**DESEVENEAK	UTP18 (Q5SSI6)	Ser 115	-	1.069	-	1.07	2.572	1.981	-	**2.28**
SRPLNAVSQDGK	YBOX3 (Q9JKB3)	Ser 328	1.076	-	1.214	1.15	1.99	-	2.047	**2.02**
SY**S**PDGKE**S**PSDK	MATR3 (Q8K310)	Ser 598 Ser 604	0.775	-	0.842	0.81	0.366	-	0.624	**0.50**
AEDGAAPSPSSE**T**PK	MARCS (P26645)	Thr 143	1.184	-	0.920	1.05	-	-	0.487	**0.49**
TVTPASSAK**TS**PAK	DPYL2 (O08553)	Thr 521 Ser 522	0.912	-	0.952	0.93	0.365	0.670	0.396	**0.48**
TA**S**PPPPPK	SRRM1 (Q52KI8)	Thr 633 Ser 635	0.958	1.084	0.959	1.00	0.363	-	-	**0.36**
VQ**S**LEGEKLSPK**S**DISPL**T**PR	MAP1B (P14873)	Ser 1768 Ser 1778 Thr 1784	0.803	0.773	0.762	0.78	0.591	0.135	-	**0.36**
SV**S**ASHEGDVK	LBR (Q3U9G9)	Ser 101 Ser 103	0.942	-	-	0.94	-	0.338	-	**0.34**
TQESCGIAPL**T**PSQSPKPEAR	HS12B (Q9CZJ2)	Thr 42	-	-	-	-	-	0.015	-	**0.02**

Supplementary Table 2 - Regulated Cell-Specific Phosphopeptides. Regulated cell-specific phosphopeptides from “Interrupted” MDA-MB-231 +DDC$^{\textit{M.tub}\text{-KDEL}}$ “Light” / C3 +Lyr$^{\text{M37-KDEL}}$ “Medium” co-cultures and “Continuous” MDA-MB-231 +DDC$^{\textit{M.tub}\text{-KDEL}}$ “Light” / C3 +Lyr$^{\text{M37-KDEL}}$ “Heavy” 5-day co-cultures (*n* = 3). Results from Control “Interrupted” MDA-MB-231 +DDC$^{\textit{M.tub}\text{-KDEL}}$ “Light” / C3 +Lyr$^{\text{M37-KDEL}}$ “Medium” and “Interrupted” MDA-MB-231 +DDC$^{\textit{M.tub}\text{-KDEL}}$ “Light” / C3 +Lyr$^{\text{M37-KDEL}}$ “Heavy” 5-day co-cultures are shown for comparison (*n* = 3). All regulated phosphopeptides are from C3 +Lyr$^{\text{M37-KDEL}}$ cells. Phosphorylated residues are underlined and bold. “Up-regulated” phosphopeptides = Heavy / Medium ≥ 2 (green) and “down-regulated” phosphopeptides = Heavy / Medium ≤ 0.5 (magenta).

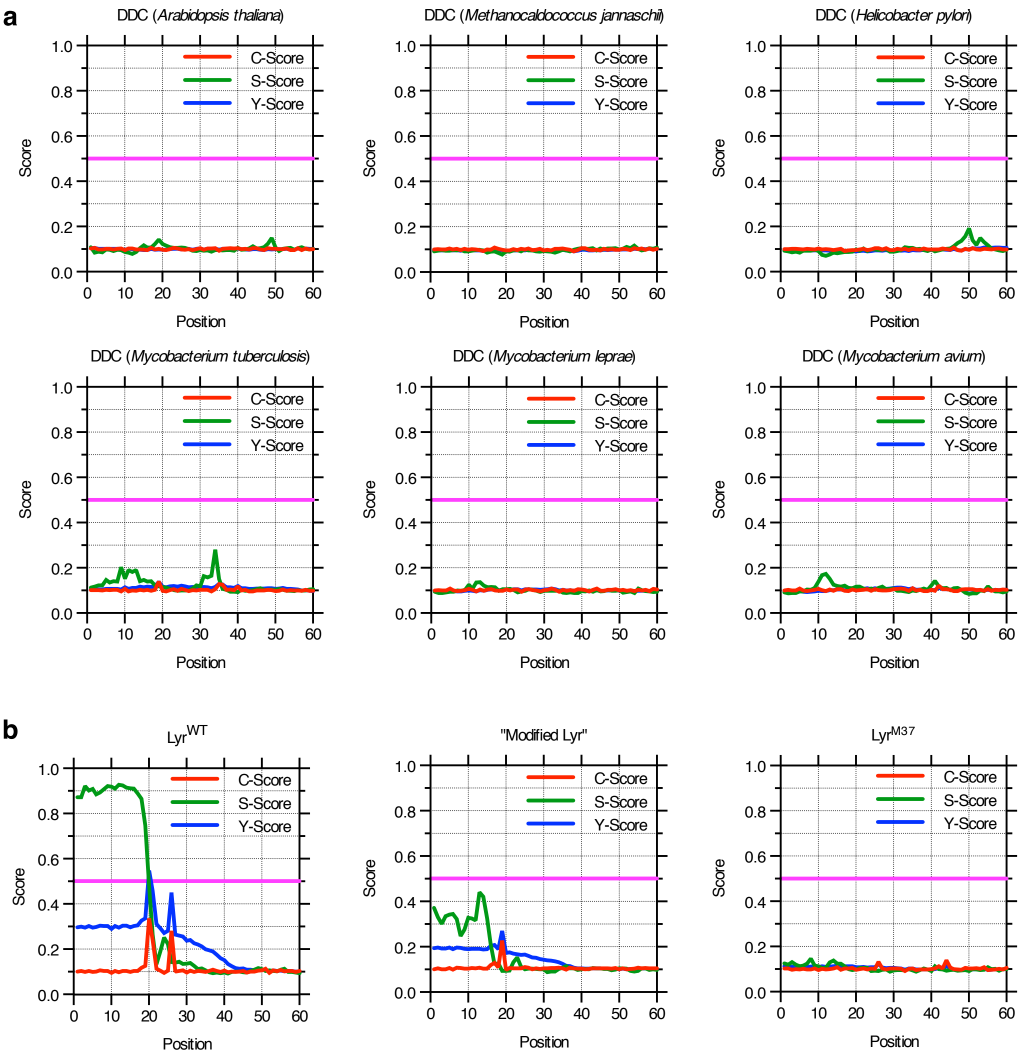
Supplementary Figure 1



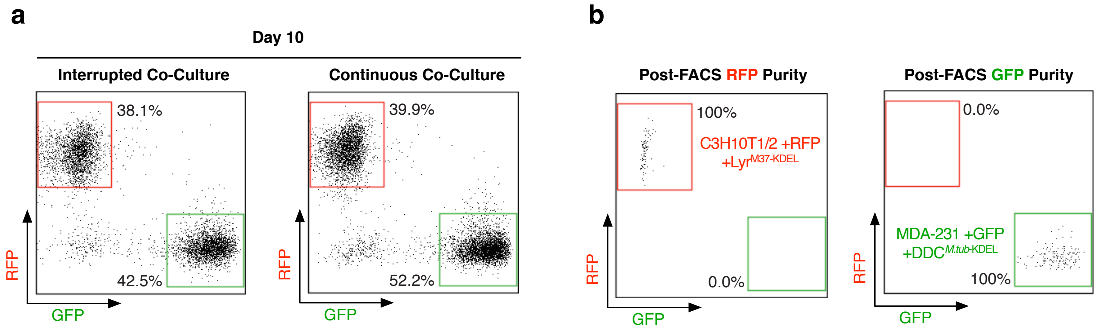
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5