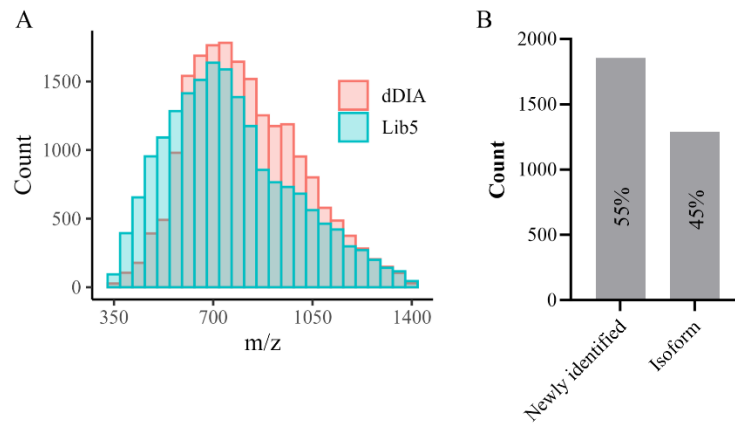
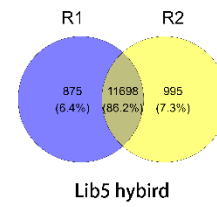
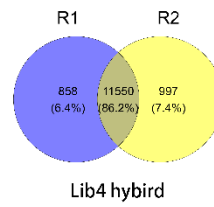
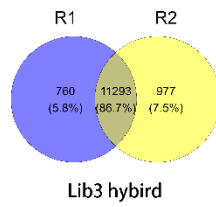
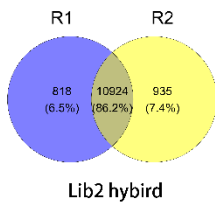
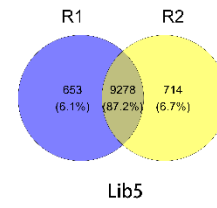
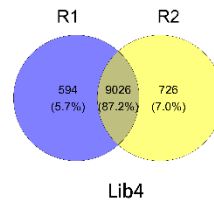
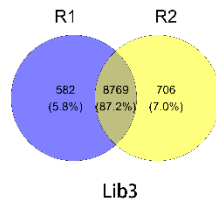
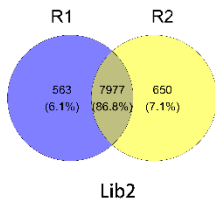
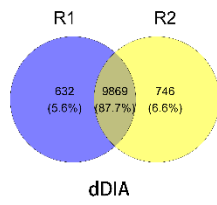


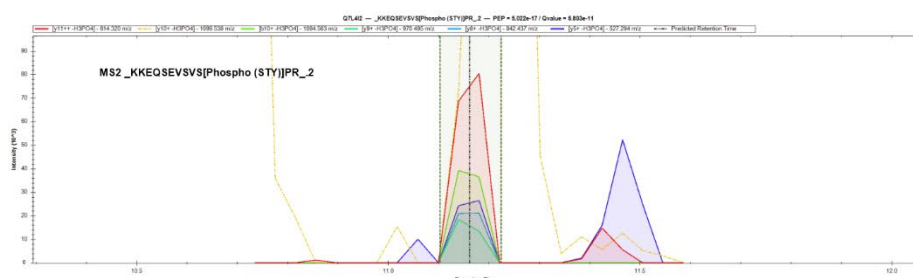
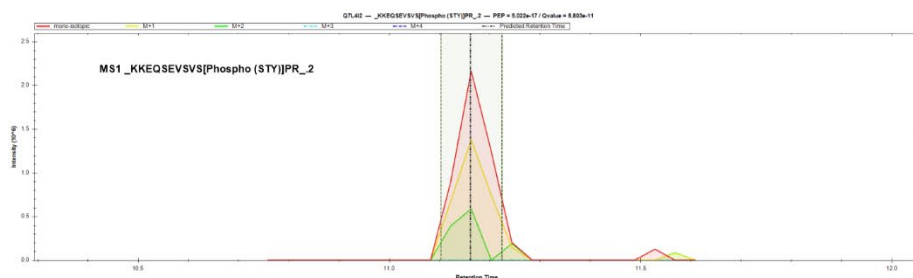
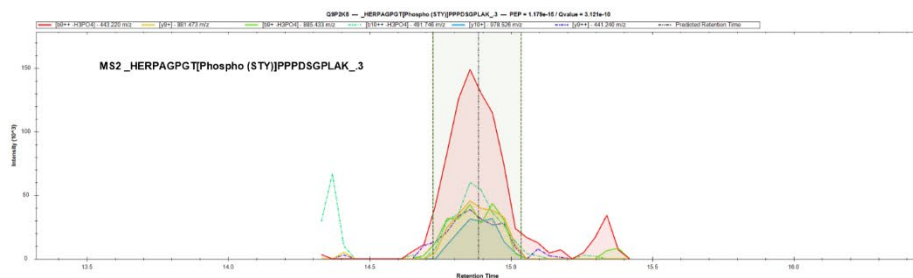
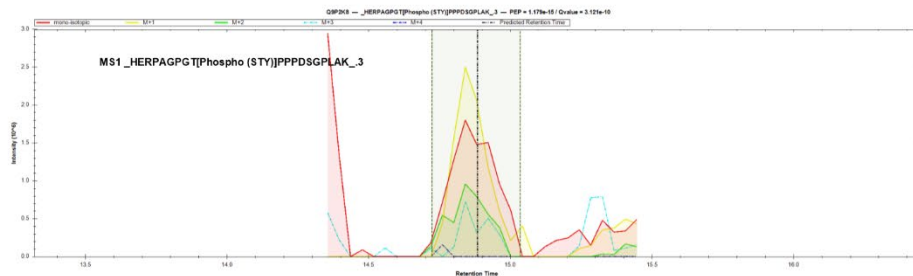
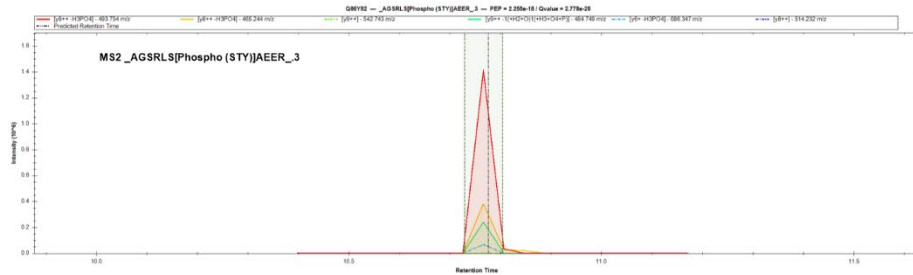
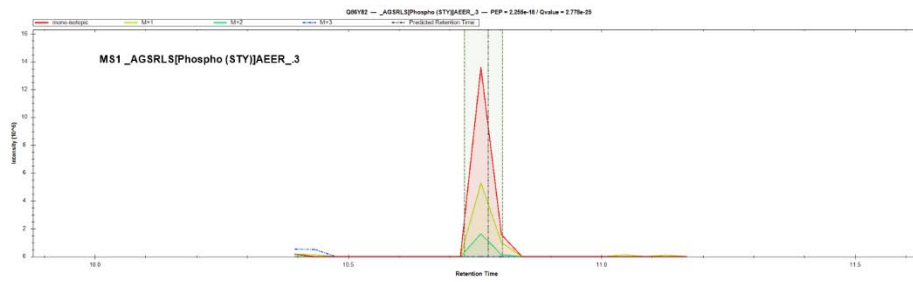
Supplementary Materials



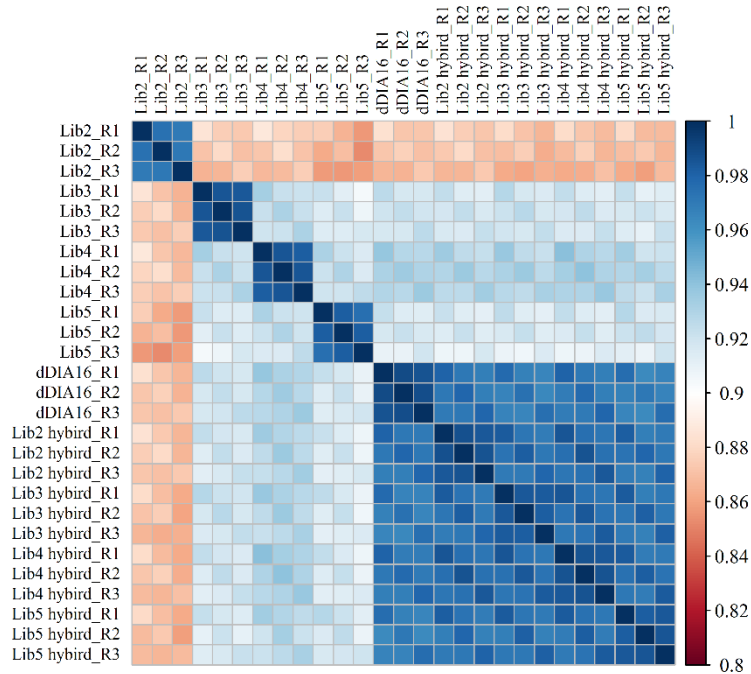
Supplementary Figure S1. Histogram of phospho-precursors identified in dDIA and lib5 based DIA (A) and bar graph of phosphopeptides identified solely in lib5 hybrid DIA (B). In B, 45% phosphopeptides were isoforms of phosphopeptides identified in direct DIA, while 55% were newly discovered.



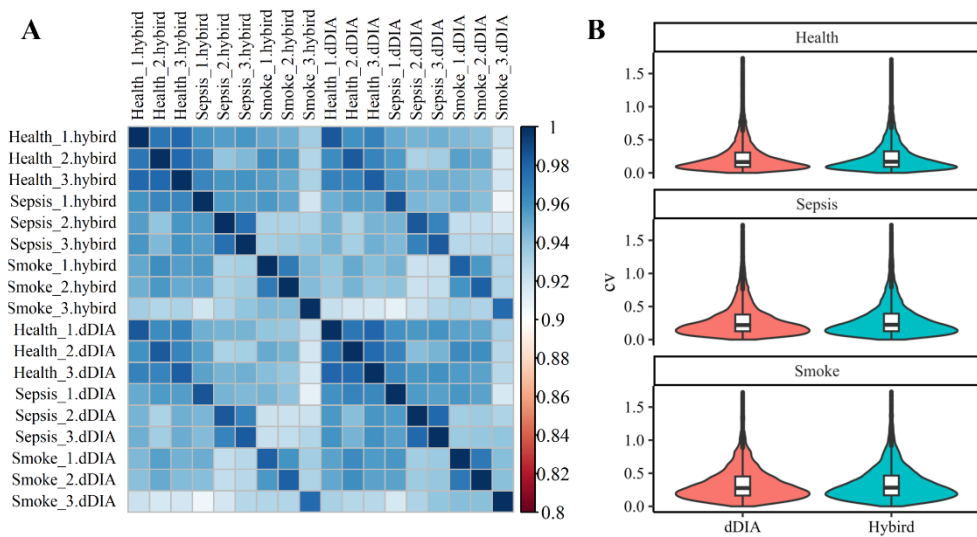
Supplementary Figure S2. Venn diagram for overlapped phosphosites between the technical replicates of direct DIA, lib2-lib5 GPF DDA base DIA and lib2-lib5 hybrid DIA.



Supplementary Figure S3. MS1 and MS2 chromatograms of three low-abundant phospho-precursors detected in hybrid DIA but not in dDIA.

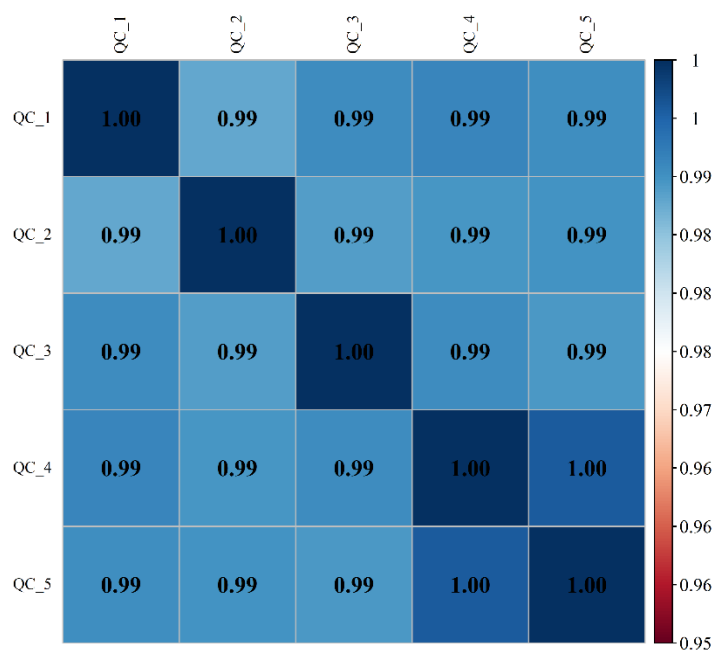


Supplementary Figure S4. Pearson correlation for phosphosites identified from direct DIA, GPF-DDA based and hybrid DIA. Three replicates were included for every search strategy.



Supplementary Figure S5. Person correlation and CVs of phosphosites identified with two DIA strategies from health lungs, as well as smoke- and sepsis-induced ALI lungs.

A. Person correlation of phosphosites. B. Violin plot of phosphosites' CVs. Boxplots were shown within the violins. dDIA, direct DIA; hybrid, GPF-DIA hybrid DIA.



Supplementary Figure S6. Person correlation of proteins from QC runs.

Supplementary Table S1. Identification summary for dDIA, Lib 2-5 based DIA and Lib 2-5 hybrid DIA.

	phospho- PSMs	phosphopeptides	phosphosites
dDIA	19685	14944	11247
Lib 2	16122	11838	9190
Lib 3	17776	13030	10057
Lib 4	18142	13386	10346
Lib 5	18732	13802	10645
Lib 2 hybrid	24027	17561	12677
Lib 3 hybrid	25182	18328	13030
Lib 4 hybrid	25755	18775	13405
Lib 5 hybrid	26353	19176	13568

Supplementary Table S2. Identification summary of standard HEK293 peptides.

	MS	MS/MS	PSMs/Purcursors	Peptides	Proteins	Median CV [%] (proteins)
QC_1	3645	71457	34048	34448	4058	
QC_2	3665	72328	35941	34596	4047	
QC_3	3595	73479	34061	33299	4023	8.7
QC_4	3532	73109	32628	33822	4013	
QC_5	3483	73931	32604	33831	4026	