SUPPLEMENTARY FIGURES



Supplementary Figure 1. Clinical significance of IncRNA AFAP1-AS1 in triple-negative breast cancer (TNBC). (A) LncRNA AFAP-AS1 expression was compared among adjacent normal tissues, TNBC tissues and non-TNBC tissues. *: *P*<0.05. (B) TNBC patients carrying low-level lncRNA AFAP-AS1 were more likely to enjoy favorable prognosis than patients with high lncRNA AFAP1-AS1 level.

Binding sites of IncRNA AFAP1-AS1 with miRNAs					
1) MiR-455-5p		11) MiR-16-5p		21) MiR-7114-3p	
Position 7775180-7775201 of IncRNA AFAP1-AS1	5'-acacauuucuuuAAGGCACAUu-3'	Position 7777266-7777287 of IncRNA AFAP1-AS1	5'-gacuggcucUGAAUUGCUGCUa-3'	Position 7779551-7779571 of IncRNA AFAP1-AS1	5'-cuuGUCUGCAUGUGUGGGUCc-3'
MiR-455-5p	3'-gcuacaucagguUUCCGUGUAu-5'	MiR-16-5p	3'-gcgguuauaAAUGCACGACGAu-5'	MiR-7114-3p	3'-gacCA CCUCUCCCCACCCAGu-5'
2) MiR-3163		12) MiR-15b-5p		22) MiR-520g-3p	
Position 7775192-7775214 of IncRNA AFAP1-AS1	5'-aaggcACAUUCACUUAUUUAUu-3'	Position 7777266-7777287 of IncRNA AFAP1-AS1	5'-gacuGGCUCUGAAUUGCUGCUa-3'	Position 7779563-7779586 of IncRNA AFAP1-AS1	5'-uguggguccgcuGGACCACUUUGg-3'
MiR-3163	3'-cagaaUG-ACGGGAGUAAAAUAu-5'	MiR-15b-5p	3'-acauUUGGUACUACACGACGAu-5'	MiR-520g-3p	3'-ugugagauuuccCUUCGUGAAACa-5'
3) MiR-370-5p		13) MiR-424-5p		23) MiR-520h	
Position 7775217-7775236 of IncRNA AFAP1-AS1	5'-agguUUGCUGCAGUGACCUa-3'	Position 7777266-7777287 of IncRNA AFAP1-AS1	5'-gacuggcucUGAAUUGCUGCUa-3'	Position 7779565-7779586 of IncRNA AFAP1-AS1	5'-uggguccgcuGGACCACUUUGg-3'
MiR-370-5p	3'-cauuGACGUCUCUGCACUGGAc-5'	MiR-424-5p	3'-aaguuuuguACUUAACGACGAc-5'	MiR-520h	3'-ugagauuuccCUUCGUGAAACa-5'
4) MiR-155-5p		14) MiR-497-5p		24) MiR-190b	
Position 7776936-7776956 of IncRNA AFAP1-AS1	5'-uaaaCAAACACAAAGCAUUAu-3'	Position 7777267-7777287 of IncRNA AFAP1-AS1	5'-acuggcucugaauUGCUGCUa-3'	Position 7779677-7779697 of IncRNA AFAP1-AS1	5'-caCUUAAGAGAUGACAUAUCg-3'
MiR-155-5p	3'-ugggGAUAGUGCUAAUCGUAAUu-5'	MiR-497-5p	3'-uguuuggugucacACGACGAc-5'	MiR-190b	3'-uuGGGUUAUAGUUUGUAUAGu-5'
5) MiR-653-5p		15) MiR-miR-6838-5p		25) MiR-miR-190a-5p	
Position 7776952-7776972 of IncRNA AFAP1-AS1	5'-auuauuuugcuaaUUCAACAa-3'	Position 7777268-7777287 of IncRNA AFAP1-AS1	5'-cuGGCUC-UGAAUUGCUGCUa-3'	Position 7779678-7779697 of IncRNA AFAP1-AS1	5'-acUUAAGAGAUGACAUAUCg-3'
MiR-653-5p	3'-gucaucucuaacaAAGUUGUg-5'	MiR-4524a-5p	3'-ucCUCAGAACGGUGACGACGAa-5'	MiR-190a-5p	3'-ugGAUUAUAUAGUUUGUAUAGu-5'
6) MiR-4524a-5p		16) MiR-4731-5p		26) MiR-2278	
Position 7777263-7777288 of IncRNA AFAP1-AS1	5'-ucuGACUGGCUCUGAAUUGCUGCUAc-3'	Position 7777277-7777298 of IncRNA AFAP1-AS1	5'-aauugcugcuacaCCCCCAGCa3'	Position 7780013-7780034 of IncRNA AFAP1-AS1	5'-cagaacucCUCAUACUGCUCUa-3'
MiR-4524a-5p	3'-acuCUGUCCAAGUACGACGAUa-5'	MiR-4731-5p	3'-gugugaguacaccGGGGGUCGu-5'	MiR-2278	3'-gguccguuGUGUGUGACGAGAg-5'
7) MiR-4524b-5p		17) MiR-512-3p		27) MiR-374c-5p	
Position 7777264-7777288 of IncRNA AFAP1-AS1	5'-cuGACUGGCUCUGAAUUGCUGCUAc-3'	Position 7777280-7777301 of IncRNA AFAP1-AS1	5'-ugcugcuacaccccCAGCACUg-3'	Position 7780034-7780057 of IncRNA AFAP1-AS1	5'-agUGCUUGG-AGGUCCGUGUAUUAc-3'
MiR-4524b-5p	3'-cuCUGUCCGAAUACG ACGAUa-5'	MiR-512-3p	3'-cuggagucgauacuGUCGUGAa-5'	MiR-374c-5p	3'-ucGUGAAUCGUCCAACAUAAUa-5'
8) MiR-892c-5p		18) MiR-216a-5p		28) MiR-655-3p	
Position 7777265-7777280 of IncRNA AFAP1-AS1	5'-ugACUGGCUCUGAAUu-3'	Position 7777284-7777307 of IncRNA AFAP1-AS1	5'-gcuacacccCCAGCACUGAGAUUa-3'	Position 7780039-7780057 of IncRNA AFAP1-AS1	5'-uuGGAGGUCCGUGUAUUAc-3'
MiR-892c-5p	3'-acUGACCGUGGAAAGACUUAu-5'	MiR-216a-5p	3'-agugucaacGGUCGACUCUAAu-5'	MiR-655-3p	3'-uuUCUCCAAUUGGUACAUAAUa-5'
9) MiR-15a-5p		19) MiR-1180-5p		29) MiR-1277-3p	
Position 7777266-7777287 of IncRNA AFAP1-AS1	5'-gaCUGGCUCUGAAUUGCUGCUa-3'	Position 7779550-7779571 of IncRNA AFAP1-AS1	5'-gcUUGUCUGCAUGUGUGGGUCc-3'	Position 7780083-7780104 of IncRNA AFAP1-AS1	5'-acgggagccACGUGUCUACGUc-3'
MiR-15a-5p	3'-guGUUUGGUAAUACACGACGAu-5'	MiR-1180-5p	3'-auAAGGGCCGGCCCACCCAGg-5'	MiR-1277-3p	3'-uuuuauguaUAUAUAGAUGCAu-5'
10) MiR-545 Position 7775206-7775227 of IncRNA AFAP1-AS1	5'-uauuuUAUUUGAGGUUUGCUGc-3'	20) MiR-195 Position 7777264-7777287 of IncRNA AFAP1-AS1	5'-cugACUGGCUCUGAAUUGCUGCUa-3'		
miR-545	3'-cguguGUUAUUUAC AA ACGACu-5'	miR-195	3'-cggUUAUAA AGAC A CGACGAu-5'		

Supplementary Figure 2. Potential sponging sites between IncRNA AFAP1-AS1 and miRNAs in accordance with the Encyclopedia of RNA Interactomes (ENCORI) online database (<u>http://starbase.sysu.edu.cn/</u>).



Supplementary Figure 3. MiRNAs potentially sponged by IncRNA AFAP1-AS1 were determined in MCF-10A, MDA-MB-231 and BT-549 cell lines (A), and luciferase activity of MDA-MB-231 and BT-549 cell lines were compared between pmirGLO-WT-AFAP1-AS1+pcDNA6.2/GW/EmGFP-miRNA group and pmirGLO-MUT-AFAP1-AS1+pcDNA6.2/GW/EmGFP-miRNA group (B). *: *P*<0.05 in comparison to pmirGLO-MUT-IncRNA AFAP1-AS1+pcDNA6.2/GW/EmGFP-miRNA group. Note: LncRNA AFAPA-AS1 fragments that contained binding sites of each miRNA were conserved and mutated, respectively, to construct WT-IncRNA AFAP1-AS1 and MUT-IncRNA AFAP1-AS1-1 for each miRNA. For each miRNA, the luciferase activity of MDA-MB-231/BT-549 cell line was compared between pmirGLO-WT-IncRNA AFAP1-AS1+pcDNA6.2/GW/EmGFP-miRNA group, both of which have been normalized to pmirGLO-WT-IncRNA AFAP1-AS1+pcDNA6.2/GW/EmGFP group.



Supplementary Figure 4. MiRNAs potentially sponged by IncRNA AFAP1-AS1 were monitored in MDA-MB-231 (**A**) and BT-549 (**B**) cell lines after silencing of IncRNA AFAP1-AS1. *: *P*<0.05 in comparison to si-negative control (NC) group.



Supplementary Figure 5. KEGG pathways enriched by genes targeted by IncRNA AFAP1-AS1-sponged miRNAs, in the light of miRPathDB online database (<u>https://mpd.bioinf.uni-sb.de/overview.html</u>).



Supplementary Figure 6. Expressions of IncRNAs were measured in 40 µmol/L formononetin-exposed MDA-MB-231 (**A**) and BT-549 (**B**) cell lines. *: *P*<0.05 in comparison to negative control (NC) group.



Supplementary Figure 7. Expressions of miRNAs were detected in MDA-MB-231 (**A**) and BT-549 (**B**) cell lines under treatment of 40 µmol/L formononetin. *: *P*<0.05 in comparison to negative control (NC) group.



Supplementary Figure 8. LncRNA AFAP1-AS1, miR-545 and miR-195 expressions were determined in triple-negative breast cancer (TNBC)-bearing mice models after injection of 80 mg/kg formononetin. *: *P*<0.05 in comparison to control group.